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# GENE EXPRESSION STUDY OF dnaK GENE IN *LACTOBACILLUS ACIDOPHILUS* BY qRT PCR IN RESPONSE TO DIFFERENT STRESS CONDITIONS

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**Abstract:** The main aim of this study was to evaluate the gene expression of dnaK gene of *Lactobacillus acidophilus* after exposed to different stress conditions. The dnak gene expression of Lactobacillus acidophilus was determined in different stress conditions such as NaCl treatment (0.2M, 0.4M, 0.6M, 0.8M and 1M) temperatures (30 °C, 35 °C, 37 °C and 40 °C). The RNA was isolated and analyzed on formaldehyde gel electrophoresis. The RNA quantity was determined by Nanodrop spectrophotometer. The cDNA was synthesized and used for Real Time PCR. The results indicate that gene expression was high in temperature 30 °C and in 1M salt stress conditions.

## INTRODUCTION

Lactic acid bacteria (LAB) are used as start up culture for production of cheese, yogurt, and fermented milk products. Lactobacilli are members of the LAB, characterized by the formation of lactic acid as a main end product of carbohydrate metabolism (Aasen, 2002). Lactobacillus comprises heterogeneous group of facultative anaerobic, catalase-negative, non-spore-forming, Gram-positive, rod-shaped organisms (Kandler, 1986). Lactobacillus can be found in various environments including milk and dairy products, plants cereals, meat and meat products fruit and vegetable as well as in gastrointestinal and urogenital tract (Mozzi, 2010, Liliana, 2006). The dairy manufacturing processes often expose LAB to adverse environmental conditions such as shifts in pH and temperature (Lorca, 2002). Tomas et al (2002) reported that *Lactobacillus* grows best in temperatures of 30-37 °C and in the presence of an inorganic carbon source (Arsene-Ploetze, 2006). Acidity is also another important factor which should be maintained in a pH range of 4.5-6.5 for optimal growth (Cai, 1999). *L. acidophilus* is a homo-fermentative microorganism having biotechnological applications (i) in the production of dairy foods and (ii) as probiotic, since it possesses the necessary characteristics to survive the harsh environmental conditions. It shows optimum growth at pH 6.0 and show wide range of acid tolerance. The probiotic properties of *L. acidophilus* include

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maintaining balance of the intestinal microflora, and treatment of acute infection. Because of the importance of this organism as probiotic, studies on its stress response mechanisms may be useful in selecting or improving *L. acidophilus* strains able to grow under harsh stress conditions (Capozzi, 2011).

Heat-shock proteins (Hsps) are molecular chaperones that play important role in bacterial stress tolerance and essential for maintenance of bacterial growth and viability. When the cell is exposed to a heat shock or other stresses, the synthesis rate of HSPs markedly increases and then slowly decreases (Singh, 2007). Hsp's play a very important role for folding of newly synthesized proteins, refolding of mis-folded proteins and transportation of proteins through biological membranes both under normal and stress conditions. DnaK is heat shock protein homolog in bacteria which transduces signals to other cellular factors when shift of temperature occurs. DnaK, DnaJ, GrpE, GroES and GroEL are important HSP's found in *L. acidophilus* (Abdullah, 2012). Probiotics used in food are exposed to adverse conditions during processing, such as temperature changes, acidity, osmotic and oxidative stress (Kosin, 2006). Such stresses may reduce the physiological activity of the cells and readily kill the cells. Once the cells have survived the stresses, they can colonize and grow to adequate numbers to provide the beneficial effect to the host. These survival mechanisms exhibited by bacteria when in stress are referred as the stress response (Khaleghi, 2012). In this study the gene expression of dnaK gene of *Lactobacillus acidophilus* was determined after exposure to different stress conditions.

## MATERIALS AND METHODS

### Bacterial strain and Stress Conditions

*Lactobacillus acidophilus* was obtained from Microbial Type Culture Collection (MTCC) and grown in de Man Rogosa-Sharpe (MRS) broth. Different stress conditions were used such as NaCl treatment (0.2M, 0.4M, 0.6M, 0.8M and 1M) and incubation temperatures (30 °C, 35 °C, 37 °C and 40 °C)

### RNA Extraction

2ml of overnight grown culture was centrifuged at 4000 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.

**Qualitative and Quantitative analysis of RNA**

The RNA was quantified by Nanodrop ND 1000 spectrophotometer. For formaldehyde gel electrophoresis 0.45g of agarose was added to 26 ml of water and heated. After cooling 3ml of 10X MOPs buffer, 0.6ml formaldehyde and 2µl of EtBr was added and gel was prepared. 10 ul of RNA sample was loaded in gel with 2µl of loading dye and ran the gel at 50V for 30 min. Bands were observed in gel doc Alpha Imager HP.

**cDNA synthesis**

For cDNA synthesis 20µl of reaction mixture was prepared in 0.2ml PCR tubes as follows. To 2µg of total RNA 2 µl of dNTP and 2µl of random hexamer was added and volume was made up to 16µl with nuclease free water. The vial was heated at 95 ˚C for 5minutes & store on ice. Then 2 µl of 10X RT buffer and 2µl of Reverse transcriptase was added and incubated at 45 ˚C for overnight. After incubation the tubes were heated at 95 ˚C for 5 min and store in -20 ˚C.

**qRT PCR:**

For Real Time PCR 10 µl of total Reaction mixture was prepared in 0.2ml PCR tubes as follows. To 1µl of cDNA product 1µl of forward primer, 1µl of Reverse primer and 5µl of SYBR Green Master Mix was added and total volume was made up to 10 ul with Nuclease free water. The PCR was set for following program-Hold @ 95 °C for 2 min, Cycling (35 repeats) Step 1 @ 95 °C hold 15 secs, Step 2 @ 60 °C, hold 30 secs.

**RESULTS AND DISCUSSION**

Using SYBR Green reagent, we determined dnaK gene expression through real-time PCR approach. SYBR green is a fluorogenic minor groove binding dye that exhibits little fluorescence when in solution but emits a strong fluorescent signal upon binding to double-stranded DNA (Morrison, 1998). The raw data fluorescence was showed in figure 1 and 2 for temp and NaCl conditions respectively.

**Figure1.** Raw data for Fluorescence for samples grown at different temperatures

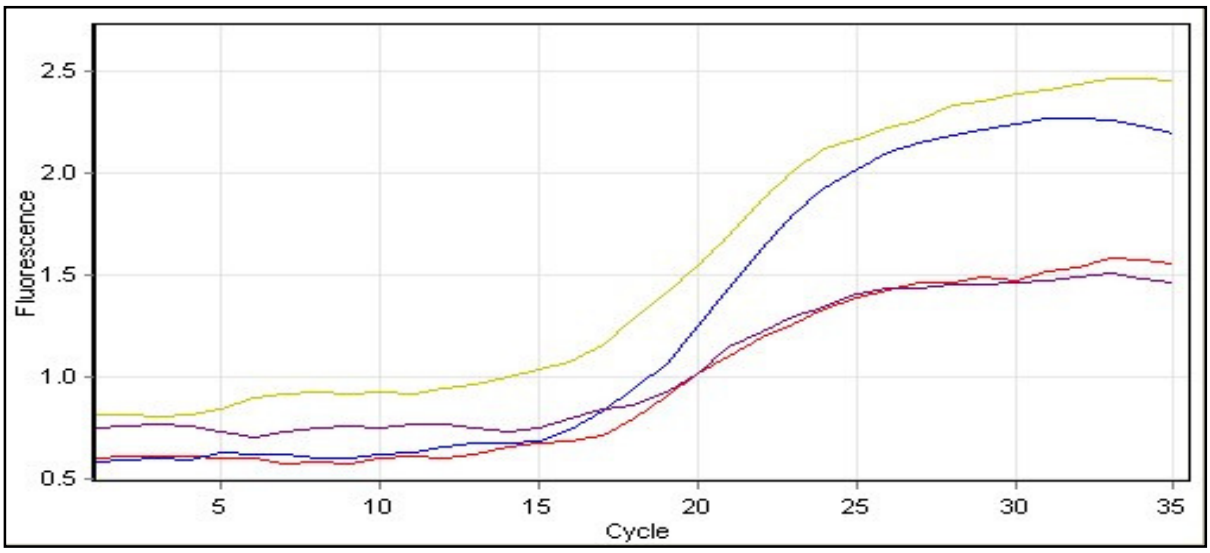
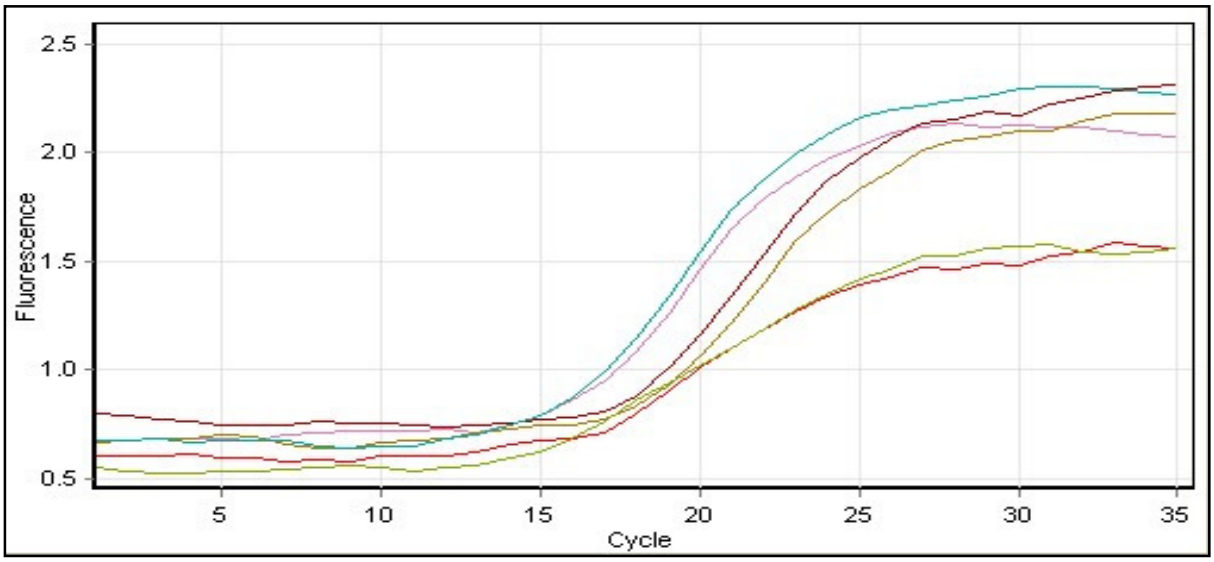



**Figure2.** Raw data for Fluorescence for NaCl Stress condition




The important parameter for quantification is the CT value. The threshold cycle or the CT value is the cycle at which the system begins to detect the increase in the fluorescent signal associated with an exponential growth of PCR product during the log-linear phase. The Threshold cycle was showed in figure 3 and 4 for temp and NaCl conditions respectively.

**Figure3.** Quantitation data for samples grown at different temperatures

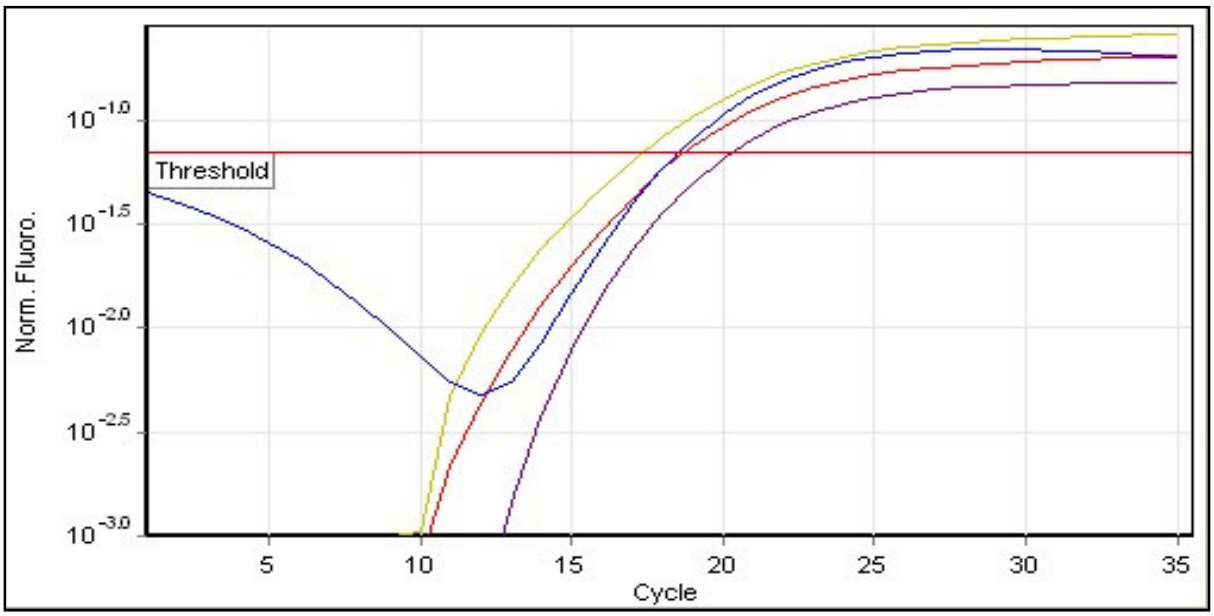
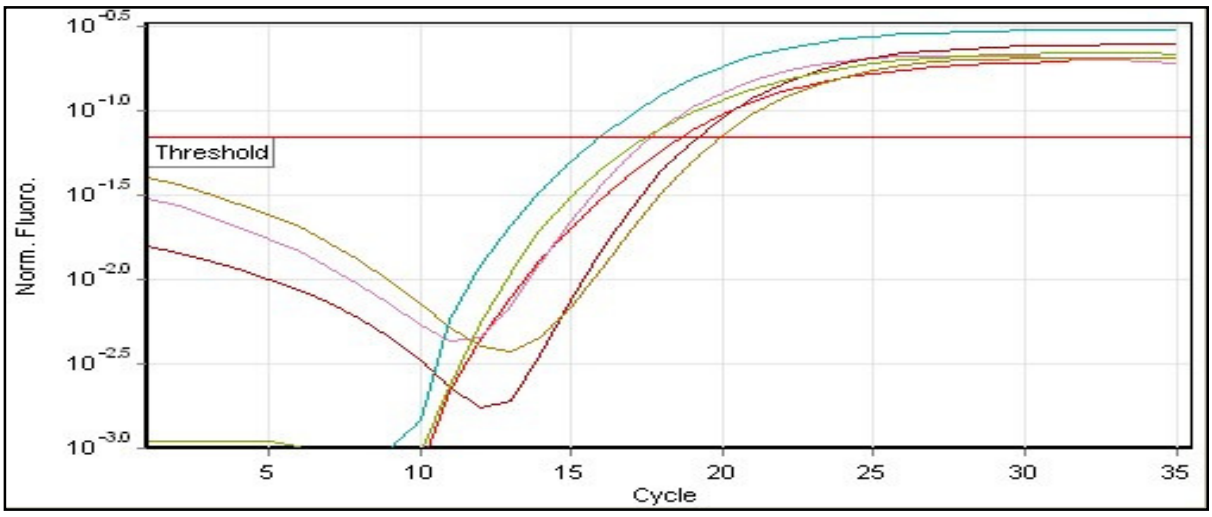



**Figure4.**Quantitation data for NaCl Stress condition




The higher the initial amount of starting DNA template, the sooner accumulated product is detected in the PCR process and the lower the CT value. The threshold should be placed above any baseline activity and within the exponential increase phase (which looks linear in the log transformation). A CT value of 40 or higher means no amplification and this value cannot be included in the calculations. Besides being used for quantitation, the CT value can be used for qualitative analysis. The CT value was showed in Table 1 and 2 for temp and NaCl conditions respectively. From the CT value the expression of dnaK gene was

determined and found highest at 30 °C. In NaCl treatment the dnaK gene expression was found maximum in 1M.

**Table1.** Ct value determination for samples grown at different temperatures

| No. | Colour | Name | Type | Ct |
|---|---|---|---|---|
| 1 | | Control | Calibrator (RQ) | 18.70 |
| 2 | | temp 30 | Unknown | 17.31 |
| 3 | | temp 35 | Unknown | 18.50 |
| 4 | | temp 37 | Unknown | 20.26 |

**Table2.** Ct value determination for NaCl Stress condition

| No. | Colour | Name | Type | Ct |
|---|---|---|---|---|
| 1 | | Control | Calibrator (RQ) | 18.70 |
| 2 | | Nacl 0.2 M | Unknown | 17.65 |
| 3 | | Nacl 0.4 M | Unknown | 19.20 |
| 4 | | Nacl 0.6 M | Unknown | 19.98 |
| 5 | | Nacl 0.8 M | Unknown | 17.50 |
| 6 | | Nacl 1 M | Unknown | 15.98 |

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