

## Original Research Article

# Expression studies of *Lactobacillus acidophilus* dnaK gene under different physical and chemical stress conditions

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## ABSTRACT

### Keywords

*Lactobacillus acidophilus*;  
dnaK; Real Time PCR;  
Gene expression.

The main aim of the present study was to evaluate the gene expression of dnaK gene of *Lactobacillus acidophilus* after exposing it to different stress conditions. The dnaK gene 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 after stress induction. The RNA quantity was determined by Nanodrop spectrophotometer. The cDNA was synthesized by reverse transcriptase and used for Real Time PCR. SYBR green is used as a dye for the quantification of double stranded DNA in quantitative PCR. The results indicate that gene expression was high in temperature 40 °C and in 1M salt stress conditions and lowest in temperature 37°C and 0.6 M salt stress conditions

## Introduction

Within the Lactic Acid Bacteria (LAB), *Lactobacillus* is of particular interest due to the fact that many members have direct impact on the health and are important for both humans and animals and *Lactobacillus acidophilus* is probably the best known species of this genus (Klaenhammer *et al.*, 2008). Approximately 80% of the yogurt manufactured contains *L. acidophilus* (Sanders, 2003). In addition to its gram positive rod shape with rounded ends, the typical size of *L. acidophilus* is 0.6-0.9 µm in width and 1.5-6.0 µm in length. Its growth condition is generally enhanced under a micro-aerophilic environment of 5% O<sub>2</sub>, 85% N<sub>2</sub>, and 10% CO<sub>2</sub> (Shah, 2006).

The optimum growth temperature is between 35°C and 40°C; it can grow at as high as 45°C. Some studies reported that the low pH is the most important factor affecting the viability of *L. acidophilus* post-fermentation (Laroia and Martin, 1991; Shah and Ravula, 2000). Ruis *et al.* (1994) pointed that *L. acidophilus* has a high cytoplasmic buffering capacity (pH 3.72-7.74), which allows it to resist changes in intracellular pH and therefore gain stability under acidic conditions. Studies on the adaptation to environmental stresses in Lactic acid bacteria (LAB) are of great interest due to the large use of these microorganisms in food industry (Klaenhammer 2005; Nga 2005).

*L. acidophilus* has been the most common Lactobacillus species isolated from the human intestine (Mitsuoka, 1992). Besides the immunomodulation, some clinical studies demonstrated the role of some probiotics such as *L. acidophilus* in lowering cholesterol (Sanders and Klaenhammer, 2001; Taranto *et al.*, 1998). In a previous report, stationary phase cells of *L. acidophilus* are acid sensitive during growth at pH 6.0 but naturally acid resistant when the pH of the culture gradually decreases during fermentation (Lorca and Font de Valdez, 2001). Molecular chaperones, which include many well studied heat shock proteins (HSP), are essential for maintenance of bacterial growth and viability (Hartl *et al.*, 1992). The most abundant and physiologically important chaperones include DnaK, DnaJ, GrpE, GroEL, and GroES. Several lines of evidence have indicated that the two major chaperone teams, DnaK-DnaJ-GrpE and GroEL-GroES, play distinct but cooperative roles in protein folding, stability, and assembly of individual proteins (Georgopoulos *et al.*, 1994). When probiotics are subjected to environmental stress, the expression of a group of proteins called heat shock proteins is upregulated. Among these proteins, DnaK and GroEL function as intracellular chaperones for denatured proteins by re-folding and assembling the proteins. Many studies have been conducted with the purpose of understanding the relationship between probiotic survival and the regulation of heat shock proteins or gene. While many investigated microorganisms, few studies used *L. acidophilus* as a model. Therefore, we studied mRNA expression of dnaK of *L. acidophilus* by using real time reverse-transcription polymerase chain reaction.

## Materials and Methods

### Induction of Stress

The *Lactobacillus acidophilus* strain used in the present study was procured from MTCC and maintained on MRS agar. Different stress conditions were studied such as NaCl treatment (0.2M, 0.4M, 0.6M, 0.8M and 1M) temperatures (30 °C, 35 °C, 37 °C and 40 °C).

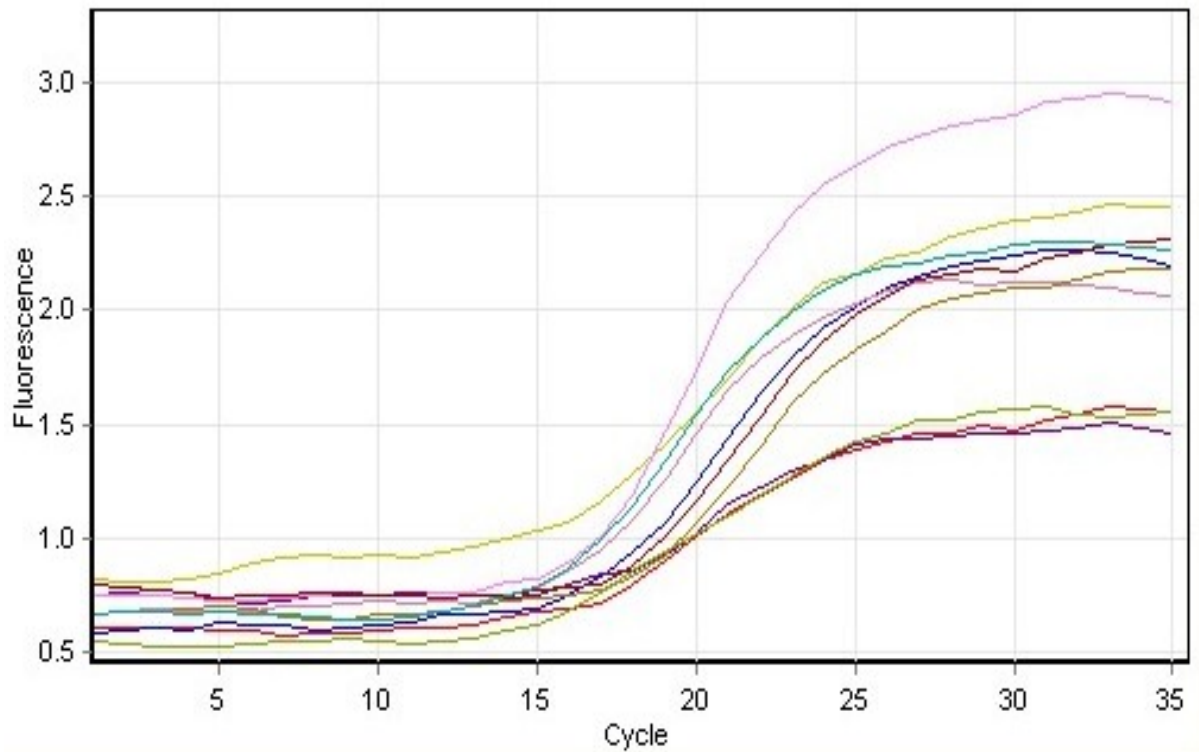
### RNA Extraction

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.

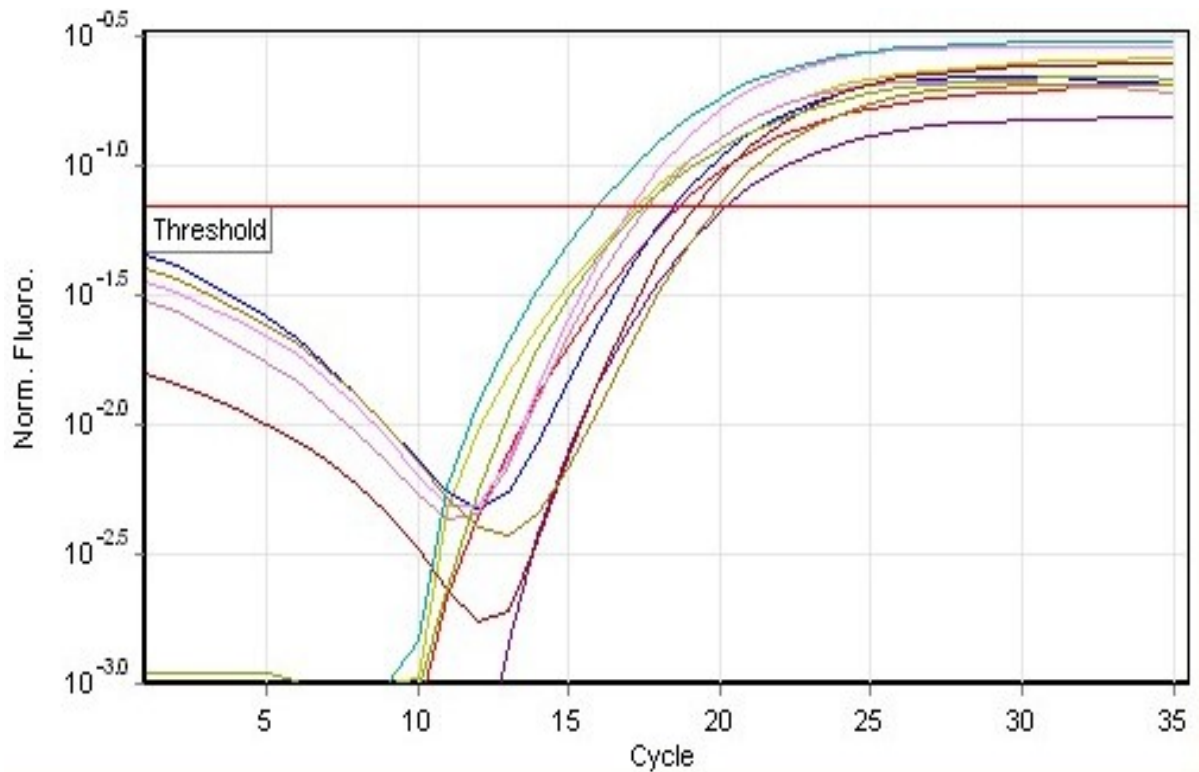
### RT-PCR

For the reverse transcriptase 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.











**Figure.1** Raw Data for fluorescence



**Figure.2** Quantitation data



**Table.1** Ct value for Temperature and NaCl treatment

S.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
5		temp 40	Unknown	17.10
19		Nacl 0.2 M	Unknown	17.65
20		Nacl 0.4 M	Unknown	19.20
21		Nacl 0.6 M	Unknown	19.98
22		Nacl 0.8 M	Unknown	17.50
23		Nacl 1 M	Unknown	15.98

## Results and Discussion

*Lactobacillus acidophilus* are considered especially as beneficial bacteria because they have their ability to break down proteins, carbohydrates and fats in food and help in absorption of necessary elements and nutrients such as minerals, amino acids and vitamins required for the survival of humans and other animals. The introduction of the new procedure based on fluorescence kinetic RT-PCR enables quantification of the PCR product in real-time. This sensitive and accurate technique measures PCR product accumulation during the exponential phase of the reaction. The technique is much faster than the previous endpoint RT-PCR as it is designed to provide information as rapidly as the amplification process itself (figure 1).

Real-time RT-PCR is a powerful tool to quantify gene expression. The quantitative endpoint for real-time PCR is the threshold cycle (CT). The CT is defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold (Figure 2). By presenting data as the CT, one ensures that the PCR is in the exponential phase of

amplification. The numerical value of the CT is inversely related to the amount of amplicon in the reaction (Table 1).

Heat shock proteins apply to a group of proteins that assist in the assembly, folding, and translocation of other proteins. In addition, they protect the cell against heat injury or other forms of stress. All cells, prokaryotic and eukaryotic, are able to respond to different cellular stresses by synthesizing these proteins. When the *L. acidophilus* is exposed to environmental stress, these stress factors (temperature, salinity) cause protein denaturation, which may affect their survival and eventually lead to cell death. To overcome these challenges, several survival mechanisms exhibited by bacteria are normally referred to as the stress response, and this response is usually associated with genes encoding a group of protein called heat shock proteins, *dnaK*.

Gene expression of *dnaK* gene was successfully determined in studied stress conditions. The expression of *dnaK* gene was found highest at 30°C and 40°C. In NaCl treatment the gene expression was found maximum and increased in 1M.

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