Comparative Protein Analysis of Heat Stress Responsiveness among different strains of *Escherichia Coli*

Vanshika Makol[1], Vardan Wadhera[2], Hemant Kardam[1], Manisha Khatri[1*]

ABSTRACT

Heat shock response is a cellular protection mechanism against unfavorable environmental conditions such as heat stress, resulting in inducible expression of heat shock proteins. The present study is designed to draw a comparative analysis between different strains of *E.coli* (DH5alpha, Rosetta, Codon+) under heat stress conditions, in terms of growth rates and protein expression. The strains were subjected to different temperature conditions (37°C, 45°C, 50°C, 55°C) for varied time periods (1hr, 3hr, 5hr, 12hr) and effect was studied in terms of OD_{600} values, which corresponds to bacterial population, and protein expression. Suppression of growth as indicated by lower OD_{600} and decreased colony numbers was observed at 55°C. Over-expression of some proteins, along with the synthesis of some new proteins was observed in all the strains at 55°C after incubation of 12h, which can be hypothesized to be heat-shock proteins. Our results could add new outlook in order to understand the importance of heat shock response at such high temperatures in *E.coli* DH5alpha.

Keywords: *Heat stress, Protein expression, Growth rate, E.coli, Strains, Heat shock proteins*

1. Introduction

Microorganisms respond to continously changing environment, temeprature change being one of the most important stress factor, by multiple adaptive mechanisms.

Environmental signals can induce dramatic changes in the expression pattern of a variety of stress-related genes, encoding proteins that improve adaptation to the changing conditions. Heat stress arising due to increased temperature, causes protein denaturation and subsequent aggregation, destabilizes macromolecules and alters membrane fluidity (Guchte *et al.*, 2002). Nowadays, for many scientific and industrial applications, the heat shock response is of great importance specifically in processes, where temperature-induced heterologous protein production takes place (Han *et al.*, 2004).

The heat shock response was discovered in 1962 by Ritossa (Ritossa, 1962) in Drosophila sp. By observing the salivary gland chomosome puffs in response to elevated temperatures. Neidhardt and Yura groups discovered the heat shock response in *E. coli* using one-dimensional or two-dimensional gels for detection of temperature-induced proteins (Lemaux *et al.*, 1978; Yamamori *et al.*, 1978)

At the molecular level, the cellular response to stress is represented by the induced synthesis of heat shock proteins (HSPs), of which molecular chaperones and proteases represent two well-characterized families of proteins. Whereas molecular chaperones function in protein folding, translocation, and refolding of intermediates, proteases, such as the ubiquitin-dependent proteasome, ensure that damaged and short-lived proteins are degraded efficiently. Temperature upshift induces expression of genes coding for heat shock proteins (HSPs). Major HSPs such as DnaK, DnaJ and GrpE, and GroEL and GroES are molecular chaperones that are important for cell survival, since they play a role in preventing aggregation and refolding proteins (Arsene *et al.*, 2000)

E. coli is able to grow over a range of approximately 40°C. The normal temperature growth range is located from 21°C to 37°C. The maximum temperature at which balanced growth can occur is approximately 49°C. *E. coli* exhibits a very high degree of both genetic and phenotypic diversity, thus is classified into many strains. Only 20% of the genes in a typical *E. coli* genome is shared among all strains. New strains of *E. coli* evolve through the natural biological processes of mutation, gene duplication, and horizontal gene transfer. The growth rate of several strains of *E. coli*, is markedly influenced in the high temperature range (40-45°C).

This study involves the comparative analysis of heat shock response at the level of protein expression in three different strains of *E.Coli*, namely DH5alpha, Rosetta and Codon+.

1. *Department of Biomedical Science, Shaheed Rajguru College of Applied Sciences for Women, University of Delhi*
2. *Amity Institute of Biotechnology, Amity University, Noida, Uttarpradesh*
* *Corresponding author* ✉ *manisha.khatri@rajguru.du.ac.in*

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2. Materials and Methods

2.1 *Bacterial strains*

Reference bacterial strain *E. coli* DH5alpha, Rosetta and Codon+ strains were obtained from Jamia Millia Islamia, New Delhi. The strains were grown in Luria Bertoni broth (LB) (Sigma-Aldrich, Missouri, USA) overnight at 37□°C. All the isolates were sub cultured regularly and stored at 4°C as well as at -80°C by making their suspension in 10% glycerol.

2.2 *Determination of growth in liquid media*

After 6 hours of growth of freshly grown *E.coli* cultures, the OD_{600} of respective culture broth was adjusted to 0.1, and 20 µl of the cultures were introduced into 20 mL of Luria-Bertani (LB) broth. For heat stress experiments, the cultures were incubated at the temperatures 37°C, 45°C, 50°C and 55°C. At the time interval of 1, 3, 5 and 12 h, the respective OD at 600 nm were measured and recorded. Besides, the respective colony forming units per mL (cfu/mL) were enumerated. Cultures were then transferred to ice water baths in order to stop cell growth, and cells were collected from 1 ml aliquots of culture. The cell pellets were frozen on dry ice and stored at −80°C.

2.3 *SDS-PAGE*

SDS-PAGE analysis of fractions was carried out in a 5–10% polyacrylamide gel gradient in standard Tris/glycine chamber buffer [0.025 M Tris/HCl (pH 8.8), 0.129 M glycine, 0.1 % SDS] at 100 V in a Mini-Protean II apparatus (Bio-Rad) (Laemmli, 1970). Broad Range (6.5–200 kDa; Bio-Rad) was used as a molecular mass standard.

3. Results and Discussion

3.1 *Effect of heat stress on cell growth*

The effect of heat stress can be most easily seen as a change in growth rate. The growth curves from experiments where *E. coli* DH5 alpha, Rosetta and Codon+ strains were subjected to heat stress at different incubation period are shown in Fig 1.It has been observed that various strains of *E. coli* are able to grow over a range of approximately 40°C and the normal temperature growth ranges from 21°C to 37°C (Luders *et al.*, 2009). Therefore in the study the growth at 37°C considered a standard growth temperature for all strains.

At 37°C, maximal and confluent growth was observed in all strains of *E.coli* at different time points. (Fig 1A.) At 45°C, growth among the three strains observed was same till 5 h of incubation period, as determined by optical

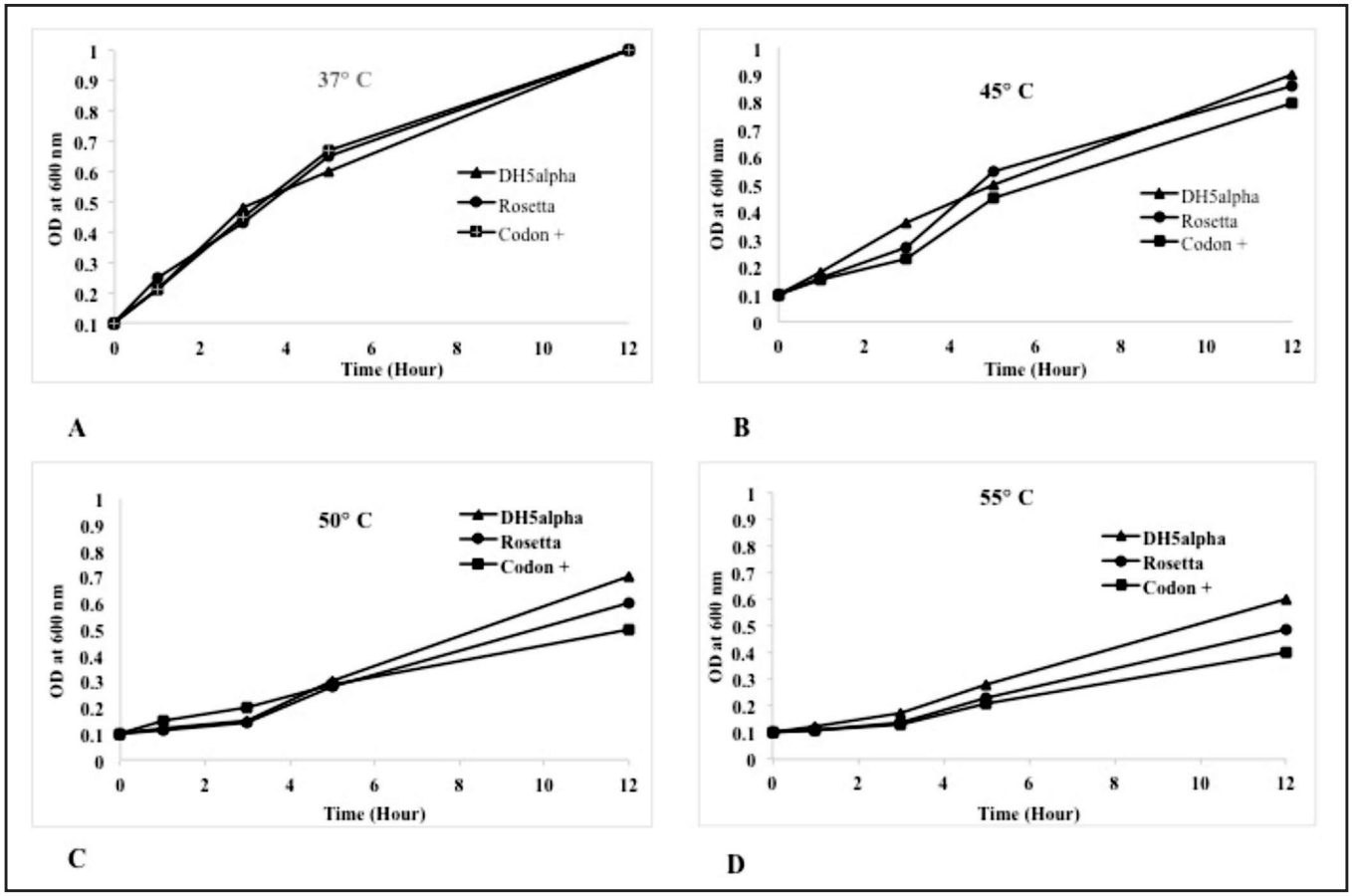

Figure 1: *Observation of cell growth in liquid media at 37°C (A), 45°C (B), 50°C (C) and 55°C (D)*

density but later on changes in growth were observed (Fig. 1B).

Further increase in the temperature by 5 °C led to slower growth in all the strains. At 50°C, although the growth of all three strains was found to be considerably slow initially in comparison to the growth at 45°C but it increased with time and after incubation of 12 hours better growth was observed (Fig. 1C). An apparently constant level of colony forming units at 37°C, 45°C and 50°C till 5 hours of incubation might be assumptive of the existence of viable but non-culturable cells.

Suppression of growth as indicated by lower OD_{600} and decreased colony numbers was observed at 55°C. After 12 hours of incubation, DH5alpha strain showed the maximum growth followed by Rosseta, and Codon+ (Fig. 1D). This might be explained by the expression of heat shock proteins in response to high temperatures. This study is unique in perspective of the survival of these strains of *E.coli* at high temperatures.

Further amplification in the level of protein expression was found when temperature was raised from 50°C to 55°C. Enhanced protein expression was observed at all the time points, but the strongest was at 12 hours (Fig. 2B), suggestive of the induction of heat shock proteins in *E.coli* in response to heat stress. The presence of these proteins in stressed bacteria is beneficial for bacterial viability during stress conditions. Their expression could be attributed to the epigenetic changes in the DNA, which helps organisms to respond accordingly to heat exposure and heat acclimation. These changes are important for survival as heat exposure greatly affects immunity, changes metabolic processes and poses a serious threat to organisms, thus heat acclimation is induced by repeated exposure to heat stress (Wu *et al.*, 2020). The adaptation of cells to heat stress leading to unfavorable changes in environmental conditions was also confirmed by the presence of additional proteins on SDS PAGE.

4. Conclusion

This study draws a comparative analysis of bacterial growth in terms of protein expression and growth rate, measured in terms of OD_{600} values, under heat stress conditions using different strains of *E.coli*. Generally, growth of laboratory strain of *E.coli* declines with the increase in temperature. This is because exposure of cells to stresses such as heat shock leads to the accumulation of partially and fully denatured proteins that interfere with normal cellular function (Noor *et al.*, 2013). In this study, we have shown that in response to heat stress (increase from 45°C to 55°C), there was 33%, 36% and 41% decline observed in the growth of DH5alpha, Rosetta and Codon+ strains respectively, as measured in terms of their OD_{600} values. At higher temperatures, the bacterial growth was overall slowed down, but a slight increase was observed after 12h of incubation in all 3 strains of *E. coli*.

The results obtained by protein expression analysis show the presence and overexpression of heat shock proteins in response to high temperatures. This overexpression was strongest at 55°C in DH5alpha, followed by Rosetta and Codon+, as indicated by band thickness on SDS-PAGE gel. Experimental studies have already proven the overexpression of heat shock proteins in maintaining the tolerance and cell viability under stress conditions (Millar *et al.*, 2012).

The results for protein expression were synonymous with the OD_{600} values. The highest OD_{600} value and maximum protein expression at 55°C in DH5alpha suggests that

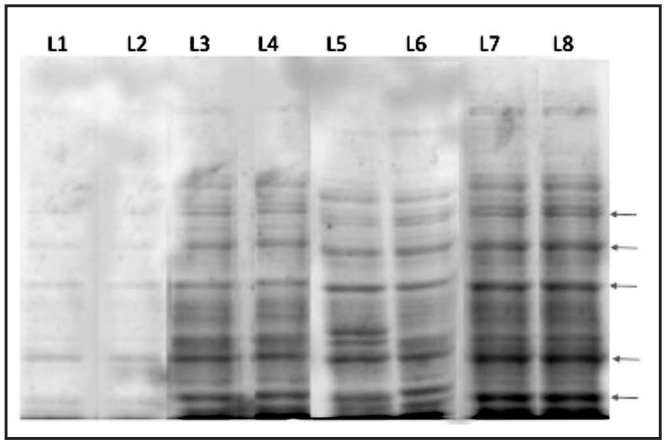

Figure 2A: *SDS-PAGE showing comparative expression of proteins (20 µl crude bacterial lysate) among different E. coli strains after heat shock stress (at 50°C after 12h of incubation) stained with Commassie blue. Duplicate sets of Lanes: L1 and L2, Control (DH5alpha at 37°C); L3 and L4, Codon+; L5 and L6, Rosetta; L7 and L8, DH5@. Arrows show overexpression of heat shock proteins.*

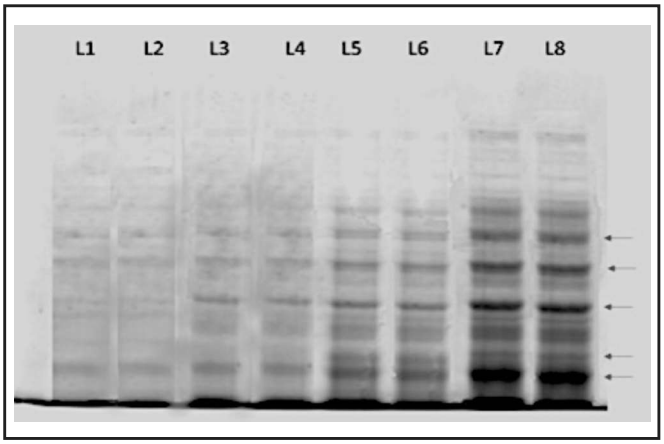

Figure 2B: *SDS-PAGE showing comparative expression of proteins (20 µl crude bacterial lysate) among different E. coli strains after heat shock stress (at 55°C after 12h of incubation) stained with Coomassie blue. Duplicate sets of Lanes: L1 and L2, Control (DH5alpha at 37°C); L3 and L4, Codon+; L5 and L6, Rosetta; L7 and L8, DH5α. Arrows show overexpression of heat shock proteins.*

DH5alpha is most heat stable, followed by Rosetta and Codon+.

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