Production of a stress protein, hsp70, by *Escherichia coli* upon exposure to a UV radiation.

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Abstract

Escherichia coli is a representative biological model for Gram-negative bacteria due to its complex cell wall structure with several DNA repair mechanisms that makes it more resilient to stress and enable to tolerate damage to its genetic material. Production of hsp70 by *Escherichia coli* bacterium upon stress was tested by exposure to a UV radiation hypothesizing that it will result in a production of significant amount of hsp70. The experimental results demonstrated a high degree variance among treatments with initial control group having the highest arbitrary value of a gene expression for hsp70.

Introduction

Heat Shock Protein 70 (hsp70), also known as a stress protein, is a small thermostable, hydrophobic protein that binds to larger unfolded proteins and assists them in folding into functional conformation. When an organism is under stress, the hsp70 detaches from heat shock factor 1 (hsf1) that it was bound to under normal conditions. This detachment activates a transcription factor, hsf1, activating the transcription of genes for heat shock proteins that protect an organism against the initial insult and produce a state of resistance to subsequent stress in the cell (Beere, et al. 2000). After an organism is subjected to a stress, levels of the hsp70 can be tested by extracting RNA from the tissue and performing a series of steps, such as RNA isolation, quantification, agarose gel and Western Blot. *Escherichia coli* (*E. coli*) is a facultative anaerobic, rod-shaped bacterium

that is commonly found in the lower intestine of endotherms and is commonly chosen as a representative biological model for Gram-negative bacteria. It could be easily and inexpensively grown in a laboratory and has been studied for decades (Alves, E., et al. 2013). *E. coli* has several DNA repair mechanisms that enable it to eliminate or tolerate damage to its genetic material (Alcantara-Diaz, et al. 2004). This experiment was designed to study a production of hsp70 by *E. coli* bacterium when exposed to stress. It is hypothesized that when *E. coli* is exposed to a UV radiation it will produce significant amounts of hsp70.

Methods and materials

Culture preparation

Non-virulent *E. coli* culture was obtained from the UW Roberts lab, from a cloning kit and spread on an Lysogenic Broth (LB) agar plate and placed in storage at 4°C after it was incubated for 16 hours at 37°C.

LB Preparation

LB was prepared in an autoclaved flask by mixing 5 g NaCl, 5 g Bactotryptone and 2.5 g Yeast Extract in a 500 mL of deionized water and homogenizing with a stir bar on a stir plate. Grown *E. coli* culture was transferred into the 50 mL of LB in two 300 mL flasks and incubated at 37°C for 16 hours and stirred on a stir plate at 75 rpm.

Samples collection

One flask was marked as Control and second marked as Treatment. 1.5 mL of E. coli control was transferred into marked tubes, centrifuged at 10000 rpm for 1 minute to pellet bacteria. Supernatant was pipetted out and pellets were frozen at -80C. Treatment flask

was placed under the stationary UV-light hood located in the Roberts Lab. At hour 1, 2 and 3 UV treatment 2 of 1.5 mL was transferred into corresponding tubes, centrifuged at 10000 rpm for 1 minute to pellet bacteria. Supernatant was pipetted out and pellets were frozen at -80C until RNA extraction.

RNA Isolation and Extraction

RNA was isolated based on the procedure from Lab 1 and extracted based on procedure from Lab 2.

RNA Quantification

RNA was quantified based on procedure from Lab 2.

Reverse Transcription

Lab 2 procedure was used for reverse transcription and procedure from Lab 5 was used for qPCR.

The following primers were used:

Forward (AE005174.2.1F -- TCCTACCAGGAAGCGATGGA) and Reverse (AE005174.2.1R -- TTTGCGGAACGCAGAAACTG)

Initially each primer was diluted as follows:

Forward --- with 325 µL nuclease free H_2O

Reverse --- with 244 µL nuclease free H_2O

Both primers were diluted even further in 1.5 mL tubes with 90 µL nuclease free H_2O.

qPCR tubes were labeled 1-10, where E_c1-0 and E_c1-3, E_c2-0 and E_c2-3 are controls at 0 and 3 hours respectively.

Arbitrary expression value calculations

Gene expression was calculated by using arbitrary expression value formula and graphed in Excel (Figure 1).

Arbitrary expression value = 10^(-(0.3012**Ct*)+11.434)

Results

Based on the calculations and graphing, control Ec1-0 at 0 hours has the highest arbitrary value 46671.10 for hsp70 by *E. coli* and E1-2 has the lowest expression 732.59 (Table 1 and Figure 1). There is a high variability in the gene expression for hsp70 among treatments.

Table 1. Experimental results with calculated arbitrary values of gene expression for hsp70 by *E. coli*, where tubes E_c1-0 and E_c1-3, E_c2-0 and E_c2-3 are controls at 0 and 3 hours respectively.

Experiment #	Efficiency	C(t)	Arbitrary Value
E_c1-0	33.56%	22.46	46671.1
E_c1-3	94.85%	26.68	2500.25
E1-1	79.75%	26.77	2348.96
E1-2	82.33%	28.45	732.59
E1-3	83.02%	27.17	1779.9
E_c2-0	64.61%	27.9	1072.8
E_c2-3	75.75%	27.01	1988.79
E2-1	78.90%	26.04	3897.19
E2-2	92.75%	24.09	15069.26
E2-3	125.62%	25.36	6245.51

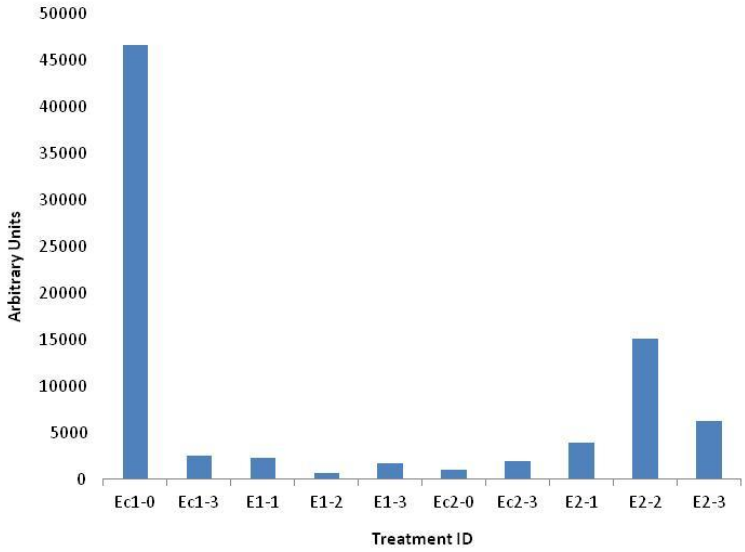

Figure 1. Comparison among treatments for gene expression (arbitrary units) for hsp70 by *E. coli*, where control E_c1-0 at 0 hours has the highest value 46671.10 and E1-2 has the lowest expression 732.59.

Discussion

The experimental results of this experiment did not coincide with the expected results, where it was expected that treatments would show higher expression of hsp70 in samples exposed to UV-treatment longer. Control E_c1-0, collected at hour 0, showed the highest gene expression for hsp70 of 46671.10 arbitrary units and treatment E1-2, collected at hour 2, exhibited the lowest value of 732.59 arbitrary units (Table 1 and Figure 1). Although it is possible for *E. coli* to adapt genetically to environmental stresses by Darwinian mutation and selection over time and exhibit a resistance to UV radiation, it is questionable if this was the case in this experiment due to the very short duration of treatment of 3 hours only (Alcantara-Diaz et al. 2004). Although, it is possible that *E. coli* had a higher hsp70 expression in a control group due to its exposure to a mechanical stress of stirring with a stir bar on a stir plate. Additional testing to compare *E. coli*'s response to UV radiation exposure and to a mechanical stress is suggested.

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