The effect of ultraviolet light on *Escherichia coli* [DNA adenine methyltransferase](https://catalyst.uw.edu/gopost/attachment/sr320/34110/DNA%20adenine%20methyltransferase) expression

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**Abstract**

**Introduction**

*Escherichia coli*, a prokaryotic bacterial cell without a membrane bound nucleus, has an adaptive response in times of stress. Horizontal gene transfer assists with changes in the phenotype, sharing genetic information across E. coli populations. Cross-stress protection has been shown to emerge as rapid and reproducible adaptation patterns, thus occupying high phenotypic plasticities within short time frames in E. coli (Dragosits et. al 2013). Resilience across strains allows for environmental persistence and an adaptive response regulated by the genotype. Therefore high diversity in *E. coli*, demonstrate the ability to express epigenetic changes in times of stress.

One enzyme that plays an important role in methalation and demethylation in E. coli is DNA adenine methylase (Dam). Dam functions in multiple cellular processes such as DNA replication, gene expression and mismatch repair (Casadesus and Low, 2006; Lobner-Olesen et al., 2005). The promoter regions for expression of Dam all include the sequence GATC and mutagenic hotspots occur close to these regions (Barras and Marinus 1989). Mutations as a result from environmental harm have the potential to allow for survival in changing conditions. Through horizontal gene transfer, mutations can be shared between prokaryotic cells.

Ultraviolet radiation is one type environmental impact that subjects cells to harm; short waves can directly cause damage to DNA. Natural responses to correct harmful rays in E coli include Dam function and regulation to correct DNA lesions or mismatch repair. By investigating the effects of UV radiation on Dam expression within E. coli, I will attempt to examine connections between harm to DNA and the increase in enzyme production to combat harm. I predict that increases in Dam enzymes will occur overtime in E. coli test groups subjected to UV radiation.

**Methods and Materials**

*Culture Growth*

An *Escherichia coli* culture was obtained from a cloning kit at the Roberts Lab in the University of Washington, Seattle. The virulence genes had been removed from the E.coli, prohibiting the transfer of hazardous bacteria. Two separate cultures were spread on agar plates containing Lysogenic Broth (LB). The plates were incubated for 16 hours at 37C then placed in storage at 4C.

To assemble the liquid LB Broth, 5g NaCl, 5 g Bactotryptone, and 2.5 g Yeast Extract were added to a sterilized container. 500mL of D/W was added then placed on a stir plate to mix. 500mL additional D/W was then added to make 1L of broth for all E. coli. experimental procedures. To grow the bacteria, swipes from the plate labeled: LB 10/28/13 SWJ E. coli were added to two sterilized beakers each containing 50mL of LB broth. The beakers were placed into the incubator for 16 hours at 37C. The stir-plate was set to 75 revolutions/minute. The experiment proceded directly after incubation.

*Experimental Setup*

The control beaker was wrapped in tinfoil then two control replicates were taken from the LB medium at time zero. The treatment was placed into a hood and the UV light was turned on. Treatment replicates were sampled at 1, 2 and 3 hours from the LB medium, for a total of 6 samples. A final control sample was taken at the 3 hour time point. Each sample was placed into the vortex for 1 minute at 10,000 rpm to pellet, and then the supernatant was removed. Pelleted samples were then transferred to storage at -80C for future RNA extraction. 

*RNA Extraction*  
 500uL of TriReagent was added to Snap cap tubes containing the 4 control and 6 treatment groups of E coli. The bacteria were homogenized using a pestle and vortexed for 5 seconds. An additional 500uL of TriReagent was added then vortexed for 15 seconds. 200uL of chloroform was added then the samples were vortexed vigorously for 30 seconds. The tubes were then left to incubate for 5 minutes. In a refrigerated microfuge, the samples were placed to spin on 14,000g for 15 minutes. The aqueous layer was pippeted of and placed into a sterile snapcap tube. 500 uL of isopropanol was added, and then inverted until fully mixed. The RNA was left to incubate at room temperature for 10 minutes, then centerfuged a 14,000 g for 8 minutes.

The supernatant was then pipetted off the RNA pellets and 1mL of 75% EtOH was added. The tubes were vortexed briefly then spun in the refrigerated microfuge at 7500 g for 5 minutes. Using a small pipette tip, the remaining supernatant was removed from the RNA pellet. The cap was left open for five minutes to let the pellet dry, and then re-suspended in 100uL of 0.1 DEPC-H20. The mixture was then incubated at 55C for 5 minutes in a hot water bath and then placed in -80C for storage. RNA was defrosted then quantified with a nanodrop spectrophotometer to test the concentration of the samples and calculated (RNA)= 40uL/mL x A360 x dilution factor.

*qPCR*

After confirming the concentration of the RNA 5ul of RNA, 0.5 uL of random primers for prokaryotes and 12.25uL of nucleate free H20 were added to a sterile PCR tube for each treatment. The mixture was left to incubate for 5 minutes at 70C in a thermocycler then transferred to ice. A combination of 5ul M-MLV 5X, 1.25uL of dNTPs, 0.5 uL of M-MLV RT and 0.5uL of nuclease free H20 was added to each PCR tube. The mixture was incubated for 60 minutes at 37C then at 70C for 3 minutes on the thermocycler. The sample was spun on a centerfuge then stored on ice at -20C.

A master mix was made, combining 50uL of SsoFast EvaGreen Supermix, 2uL of 10x diluted upstream DNMT primer 5' GCAACTGAAGCGTCAGCAAA 3' , 2uL 10x diluted downstream DNMT primer 5'CCGCATAACGCAACTGATGG and 42uL of ultra pure water. 24uL of the master mix was added to 4 white PCR wells. 1uL of the cDNA was placed in 2 reaction wells; 1uL of ultra pure water was added to the blank wells. Clean lids were placed on the tubes, and then loaded into the PCR machine for the following conditions: 95°C for 10 minutes, 95°C for 15s, 55 °C for 15 s, 72°C for 15 s (+ plate read). The Procedure was repeated from step two then repeated 39 more times. Finally the PCR machine tempered at 95°C for 10s, then processed the melt curve from 65°C to 95°C, at 0.5°C for 5s (+plate read)

**Results**

Expression of [DNA adenine methyltransferase](https://catalyst.uw.edu/gopost/attachment/sr320/34110/DNA%20adenine%20methyltransferase" \o "Dna adenine methylase) in *Escherichia coli* was measured using qPCR on Treatment samples exposed to UV radiation and control groups that were not exposed to any light.   
Arbitrary expression value was determined by using C(t) values obtained from qPCR and the formula =10^(-(0.3012\**Ct*)+11.434). Results are listed in Table 1.

State results for melting curve and negative control.

Table 1. qPCR results for [DNA adenine methyltransferase](https://catalyst.uw.edu/gopost/attachment/sr320/34110/DNA%20adenine%20methyltransferase" \o "Dna adenine methylase) expression in *Escherichia coli*. Treatment groups were exposed to Ultraviolet radiation for 3 hours and samples were withdrawn each hour to determine response. The arbitrary expression value was determined as value =10^(-(0.3012\**Ct*)+11.434)

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Efficiency | C(t) | Arbitrary Expression Value |
| Control 1 Hour 0 | 216.77% | 17.96 | 1057908 |
| Treatment 1 Hour 1 | 136.47% | 19.54 | 353632 |
| Treatment 1 Hour 2 | 493.93% | 22.99 | 32316 |
| Treatment 1 Hour 3 | 189.81% | 21.23 | 109528 |
| Control 1 Hour 3 | 90.16% | 18.73 | 620189 |
| Control 2 Hour 0 | 146.45% | 20.63 | 166052 |
| Treatment 2 Hour 1 | 145.75% | 20.73 | 154926 |
| Treatment 2 Hour 2 | 178.46% | 22.19 | 56282 |
| Blank | N/A | N/A | Blank |
| Treatment 2 Hour 3 | 149.27% | 18.38 | 790576.7 |
| Control 2 Hour 3 | 126.74% | 24.08 | 15174.14 |
| Sample | 37.36% | 34.98 | Blank |

Figure 1. Mean [DNA adenine methyltransferase](https://catalyst.uw.edu/gopost/attachment/sr320/34110/DNA%20adenine%20methyltransferase) expression in *Escherichia coli*. Treatment groups were subjected to ultraviolet radiation exposure for 3 hours. Samples were removed each hour and qPCR was run on bacterial RNA to determine arbitrary values of expression.

**Discussion**

References:

Barras, F. & Marinus, M.G., 1989. The great GATC: DNA methylation in E. coli. *Trends in Genetics*, 5, pp.139–143. Available at:<http://www.sciencedirect.com/science/article/pii/0168952589900541>[Accessed November 30, 2013].

Casadesús, J. & Low, D., 2006. Epigenetic Gene Regulation in the Bacterial World. *Microbiology and Molecular Biology Reviews*, 70(3), pp.830–856. Available at:<http://mmbr.asm.org.offcampus.lib.washington.edu/content/70/3/830>[Accessed November 30, 2013].

Dragosits, M. et al., 2013. Evolutionary potential, cross-stress behavior and the genetic basis of acquired stress resistance in Escherichia coli.*Molecular Systems Biology*, 9, p.643. Available at:<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3588905/> [Accessed November 30, 2013].

Herman, G.E. & Modrich, P., 1982. Escherichia coli dam methylase. Physical and catalytic properties of the homogeneous enzyme. *Journal of Biological Chemistry*, 257(5), pp.2605–2612. Available at:<http://www.jbc.org/content/257/5/2605> [Accessed November 30, 2013].

Lobner-Olesen, A., Skovgaard, O., Marinus, M.G., 2005. Dam methylation: coordinating cellular processes. Curr. Opin. Microbiol. 8, 154–160.

Palmer, B.R. & Marinus, M.G., 1994. The dam and dcm strains of Escherichia coli — a review. *Gene*, 143(1), pp.1–12. Available at:<http://www.sciencedirect.com/science/article/pii/0378111994905975>[Accessed November 30, 2013].

Sun, K. et al., 2010. DNA adenine methylase is involved in the pathogenesis of Edwardsiella tarda. *Veterinary Microbiology*, 141(1–2), pp.149–154. Available at:<http://www.sciencedirect.com/science/article/pii/S0378113509004052>[Accessed November 30, 2013].

Urig, S. et al., 2002. The Escherichia coli Dam DNA Methyltransferase Modifies DNA in a Highly Processive Reaction. *Journal of Molecular Biology*, 319(5), pp.1085–1096. Available at:<http://www.sciencedirect.com/science/article/pii/S0022283602003716>[Accessed November 30, 2013].