The Impact of DNA Methyltransferase on Bacterial Growth in *E. coli*

Jessica LoPresti and Kevin T. Militello
Biology Department, SUNY Geneseo

Abstract

The Dcm protein (DNA cytosine methyltransferase) catalyzes the process of DNA methylation, a process that has a large role in the regulation of gene expression in cells. The Dcm protein methylates at the second C at the 5'CCWGG3' site. The specific consequences of this methylation are not known. We have been studying DNA methylation in *E. coli* by using two different measures of growth. The first experiment was a growth curve using absorption spectrophotometry of wild-type *E. coli* and *E. coli* with a *dcm* knockout gene at a temperature stressor of 42°C. We tracked the growth over eight hours, after first growing cultures at 37°C since the bacteria had no difference in growth at that temperature. The second experiment used the same methods, but instead of the wild-type bacteria, a *dcm* knockout strain with a Dcm plasmid added back in via genetic complementation was used. We also plated overnight cultures of the wild-type *E. coli* and *dcm* knockout strains to utilize another mechanism to measure growth. It was found that the wild-type *E. coli* strain grew at the fastest rate of the four strains. This raises some questions regarding the significance of the *dcm* gene, as the bacteria grows fastest when the *dcm* gene has been present in the protein from start to finish. If the *dcm* gene can withstand the high temperature stressors, we may be able to explore how the protein in bacteria may react to other stressors, and dissect possible medical and pharmaceutical implications.

Introduction

- *Dcm* methylates at the second C at the 5'CCWGG3' site, generating 5-methylcytosine.
- It plays an important role in gene expression, but shows few phenotypic effects, such as changes in sensitivity to ethidium bromide and expression only during the stationary phase of Dcm.
- Our research explores the impact of high temperature stress on bacterial growth using Wild-type and *dcm* knockout strains of *E. coli.*

Methods

Growth Curve

Overnight cultures grown at 37°C

- WT — Wild-type strain
- Δ*dcm* — *dcm* knockout strain
- JW-21 — *dcm* knockout strain with plasmid containing functional *dcm* gene
- JW-9 — *dcm* knockout strain with plasmid containing truncated *dcm* gene

Put strains in incubator at 42°C
Spectrophotometry at 600 nm
-triplicates averaged
-measured every hour over 8 hours

Bacterial Assay

WT | Δ*dcm*

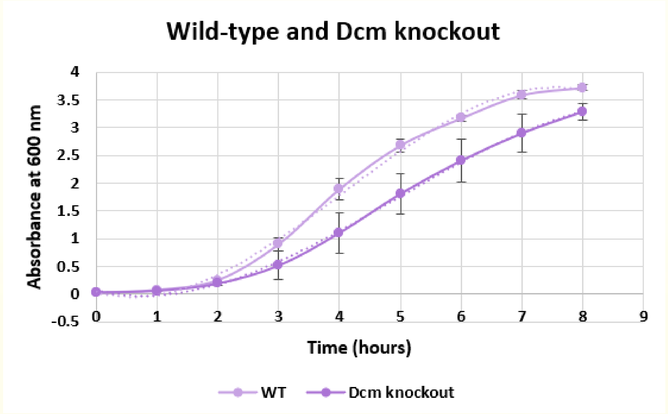
Overnight cultures grown at 37°C

Diluted with saline onto LB plates for dilutions:
1e-4, 1e-5, 1e-6, 1e-7

Colonies counted and convert back to CFU/mL

Results

A



B

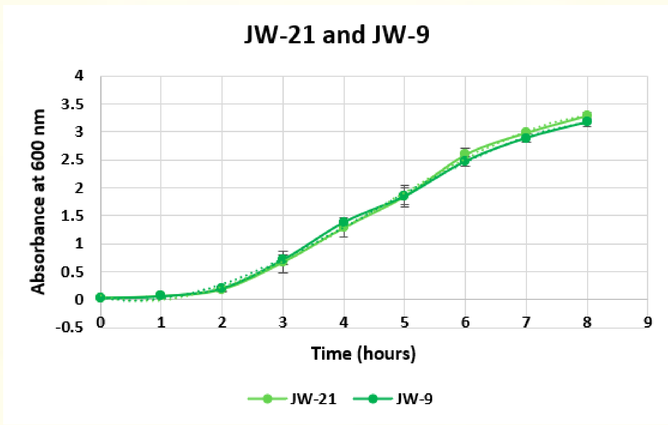
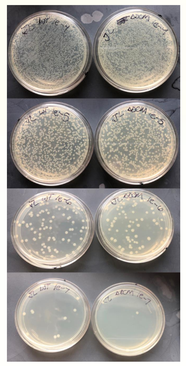


Fig. 1a and 1b. *Comparison of experiment with WT and dcm knockout spectrophotometry over time and JW-21 and JW-9 spectrophotometry over time*. Overnight cultures of the four strains were grown at 37°C, then incubated at 42°C. Spectrophotometry at 600 nanometers was used to measure growth (n=3). The Wild-type *E. coli* grew the fastest in comparison to the three other strains tested. WT and *dcm* knockout are shown in purple in the upper graph, and JW-21 and JW-9 are shown in green on the lower graph.



Temp.	Strain	CFU/mL
37°C	WT	$1.98*10^9$
37°C	Dcm knockout	$1.52*10^9$

Fig. 3. *Assay of wild-type (left) and* dcm *knockout (right) strains on LB plates*. Overnight cultures of the Wild-type and *dcm* knockout strains were grown at 37°C, then diluted and plated. This time, there was more growth on the wild-type plates (n=1) but further experiments need to be performed.

Conclusion

Genetic complementation was used to determine how the *dcm* gene affected growth at a high temperature. We can't draw a firm conclusion that it was the gene, because the JW-21 strain grew at a similar rate to the *dcm* knockout and JW-9 strains. There is a number of external factors that could explain why the JW-21 strain may have not grown faster than the JW-9 strain, unrelated to the *dcm* gene.

Future Directions

For now, we plan to continue the bacterial assays at higher temperatures than 37°C to get a better indication if high temperature has an effect on growth in *E. coli* due to the *dcm* gene. If we can figure out the impact the *dcm* gene has on growth, we can hypothesize what else the *dcm* gene can have an effect on in the human body, and uncover possible health implications the gene may be responsible for.

References

Militello KT, Finnerty-Haggerty L, Kambhampati O, Huss R, Knapp R. DNA cytosine methyltransferase enhances viability during prolonged stationary phase in *Escherichia coli*. FEMS Microbiol Lett. 2020 Nov 5;367(20):fnaa166. doi: 10.1093/femsle/fnaa166. PMID: 33045036.

Militello KT, Simon RD, Mandarano AH, DiNatale A, Hennick SM, Lazatin JC, Cantatore S. 5-azacytidine induces transcriptome changes in *Escherichia coli* via DNA methylation-dependent and DNA methylation-independent mechanisms. BMC Microbiol. 2016 Jun 27;16(1):130. doi: 10.1186/s12866-016-0741-4. PMID: 27349222; PMCID: PMC4924334.

Militello KT, Mandarano AH, Varechtchouk O, Simon RD. Cytosine DNA methylation influences drug resistance in *Escherichia coli* through increased sugE expression. FEMS Microbiol Lett. 2014 Jan;350(1):100-6. doi: 10.1111/1574-6968.12299. Epub 2013 Nov 6. PMID: 24164619.

Militello KT, Simon RD, Qureshi M, Maines R, VanHorne ML, Hennick SM, Jayakar SK, Pounder S. Conservation of Dcm-mediated cytosine DNA methylation in *Escherichia coli*. FEMS Microbiol Lett. 2012 Mar;328(1):78-85. doi: 10.1111/j.1574-6968.2011.02482.x. Epub 2012 Jan 6. PMID: 22150247; PMCID: PMC3976566.

Acknowledgements

I would like to thank Dr. Militello and the SUNY Geneseo Biology Department for the continued support, guidance and assistance in the lab.