

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

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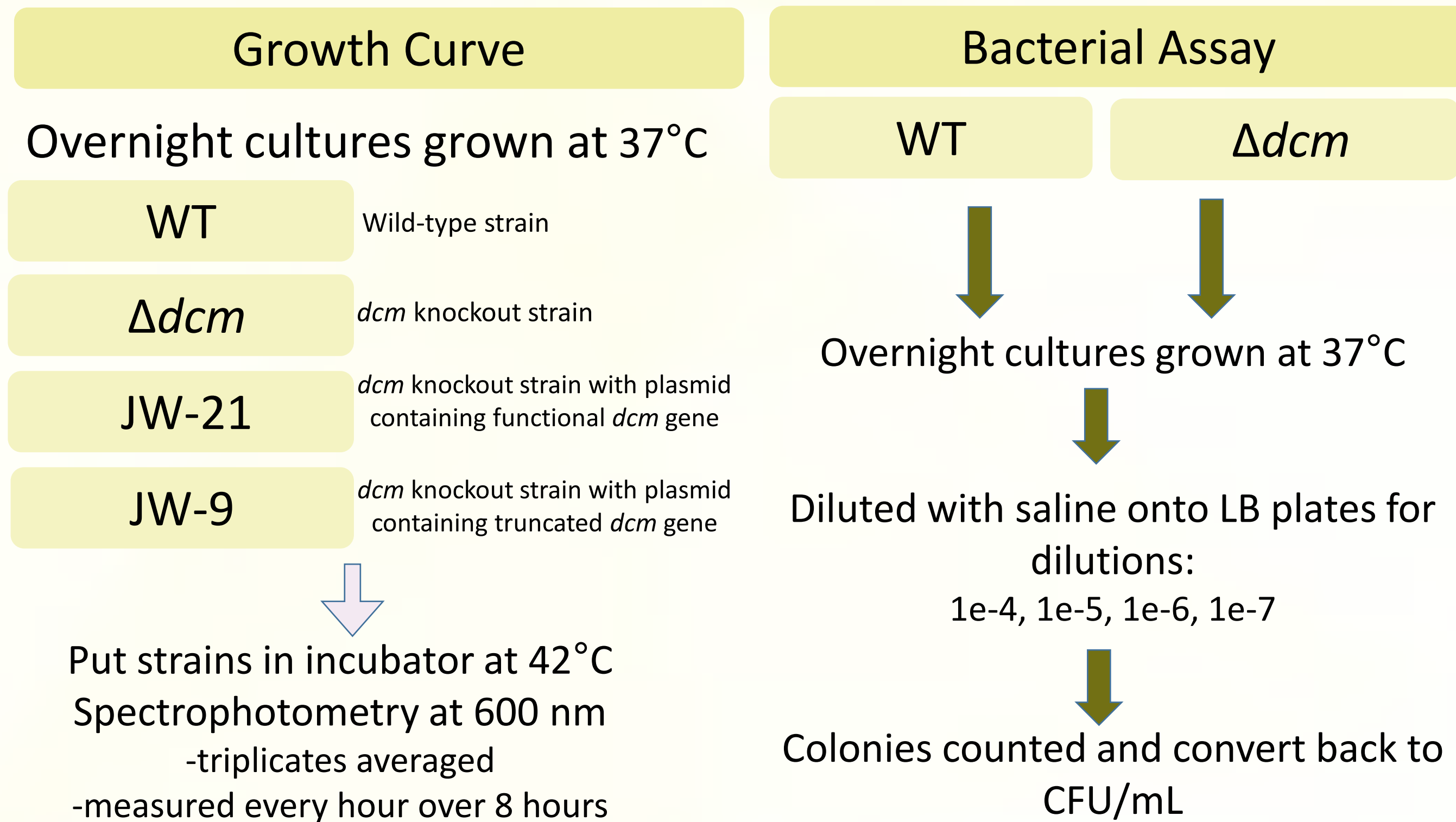
Abstract

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



Results

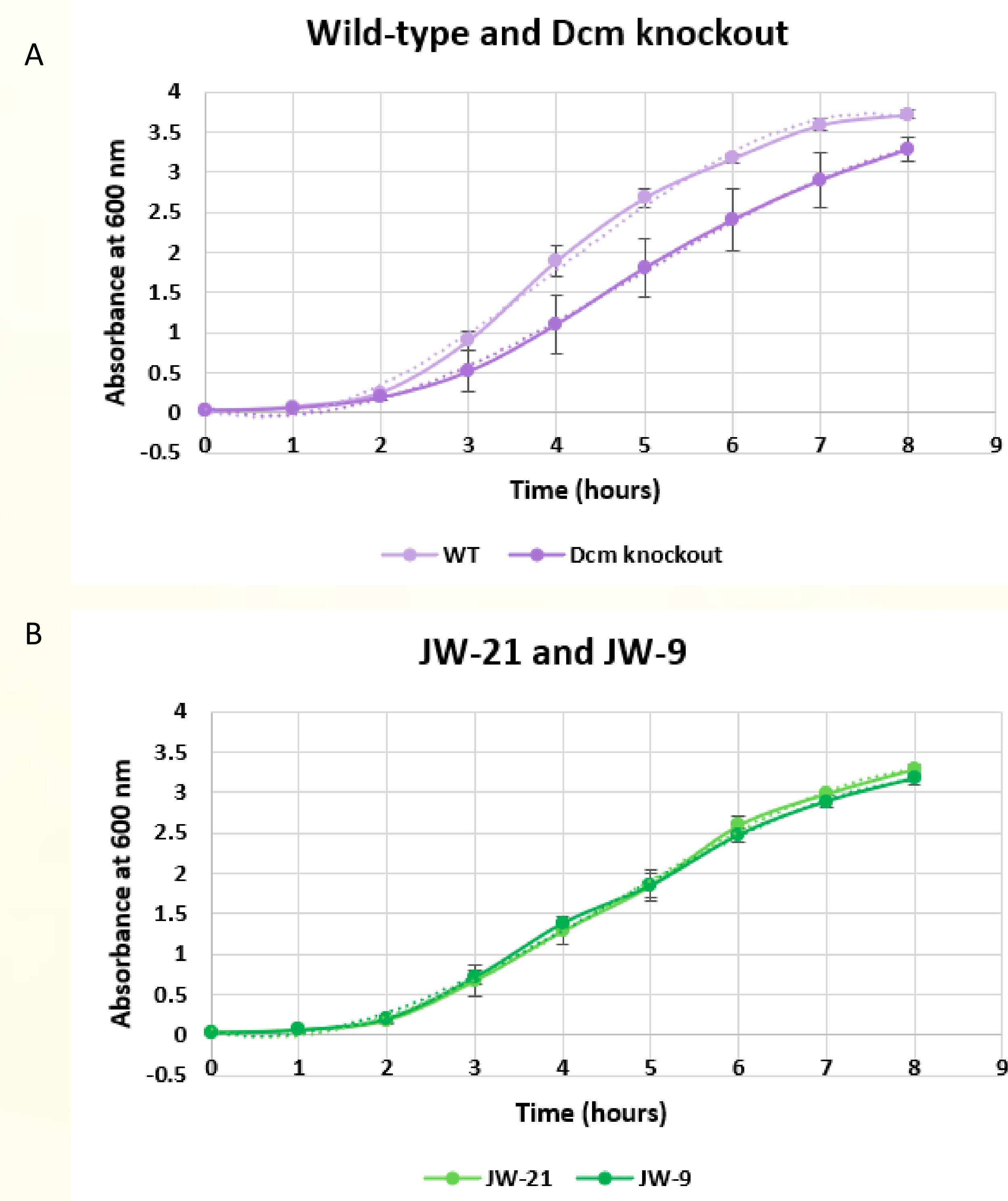


Fig. 1a and 1b. Comparison of experiment with WT and *dcm* knockout spectrophotometry over time and JW-21 and JW-9 spectrophotometry over time. Overnight cultures of the four strains were grown at 37°C, then incubated at 42°C. Spectrophotometry at 600 nanometers was used to measure growth (n=3). The 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.

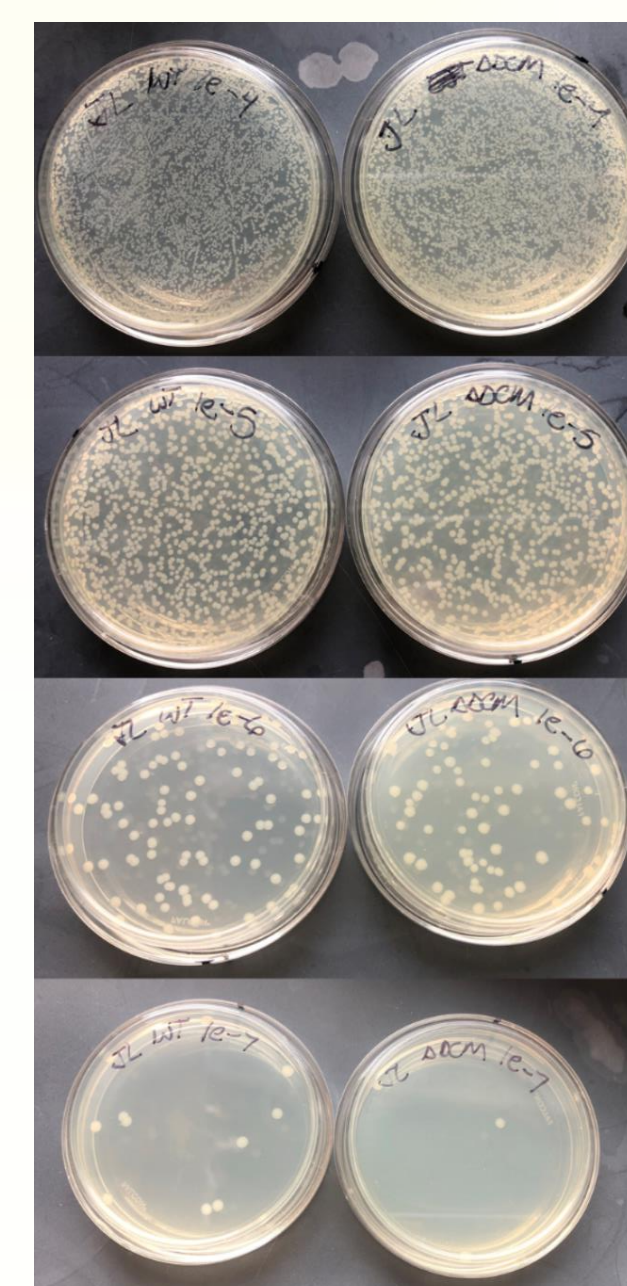


Fig. 3. Assay of wild-type (left) and *dcm* knockout (right) strains on LB plates. Overnight cultures of the Wild-type and *dcm* knockout strains were grown at 37°C, then diluted and plated. This time, there was more growth on the wild-type plates (n=1) but further experiments need to be performed.

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

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

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Acknowledgements

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