The Role of the *trmB* Gene on the Morphology and Growth of *Haloarcula hispanica*

Kevin Chen, Lucy Grossmann, Elayna Lei, Sahil Patel, Rachel Qu, Andrea Schwartz

North Carolina School of Science and Mathematics

Introduction

Haloarcula hispanica is a halophilic species of archaea; not much is known about how the species functions. Previous studies indicate that *Hca. hispanica* contains the transcription factor TrmB which regulates metabolic processes through activation of gluconeogenesis and inhibition of glycolysis [1]. Investigation into TrmB in the *Halobacterium salinarum* archaea concluded that the inactivation of TrmB leads to a change in its shape and growth rate [1].

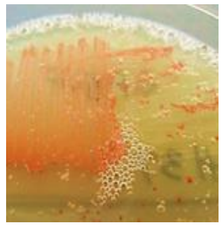

Figure 1.1. Plated *Hca. hispanica.*

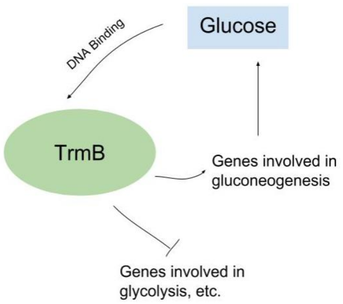

Figure 1.2. Regulation pathway for TrmB protein.

Based on this research, wild type and Δ*trmB* strains of *Hca. hispanica* were observed to determine the effects of the *trmB* gene on the shape and growth rate of the cells in the presence of glucose.

How does the *trmB* gene affect the structure and growth of the archaeon *Haloarcula hispanica* through varying conditions?

We hypothesized that in the absence of glucose, the Δ*trmB* strain would grow significantly less than the wild-type strain and the shape would be different.

Methods

We first observed the shape of *Hca. hispanica* under different conditions by utilizing a phase-contrast microscope.

Then, the wild-type and Δ*trmB* strains were aliquoted to 64 wells at 37 °C. They were equally divided into either filtered or unfiltered media receiving a proportionate amount of a 0.075% glucose solution. To reduce error, 4 biological replicates and 2 technical replicates were implemented per condition and strain. Optical density measurements were collected using a bio-screen over a 48 hour period.

Microscopy of Wild Type & ΔtrmB

No significant difference was found by the Student's t-test (p=0.704) in the length to width ratios of the wild type *Hca. hispanica* and the mutant Δ*trmB* when observed at 100x magnification.

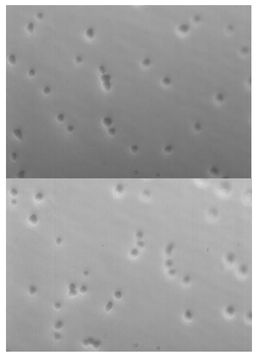

Figure 2.1. Wild Type (top) compared to Δ*trmB* mutant (bottom) at 100x magnification.

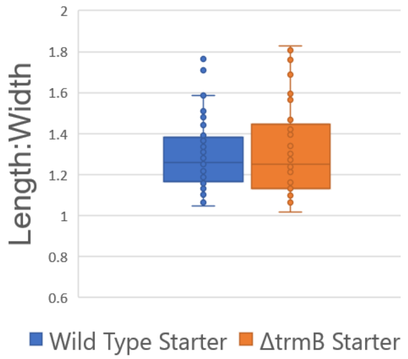

Figure 2.2. Wild-type and Δ*trmB* cell length/width ratio with error bars of one standard deviation.

Average Growth Curves in Different Conditions

Δ*trmB* with no glucose is shown to follow a linear pattern, demonstrating little to no growth. With glucose, the growth of Δ*trmB* matches the wild type. The filtered area under the curve (AUC) for both strains is less effective than the unfiltered AUC, as the archaea grows better in an unfiltered media. This can be attributed to unidentified particulates that occurred in the unfiltered media.

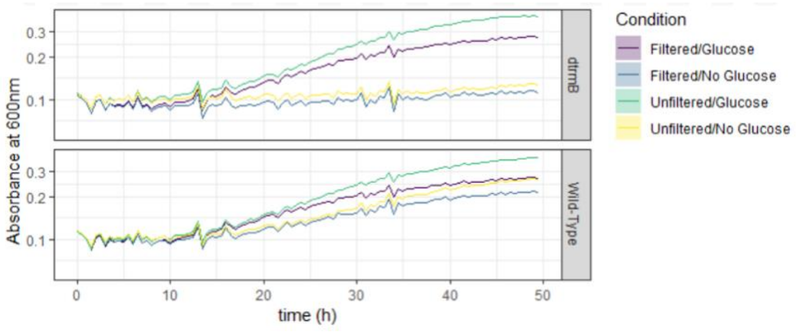

Figure 3. Growth of *Hca. hispanica* Wild-Type and Δ*trmB* strains in different conditions. Most of the growth curves follow the general trend of a lag phase, a maximum growth rate, and an asymptotic phase [2].

Maximum Growth Rates in Different Conditions

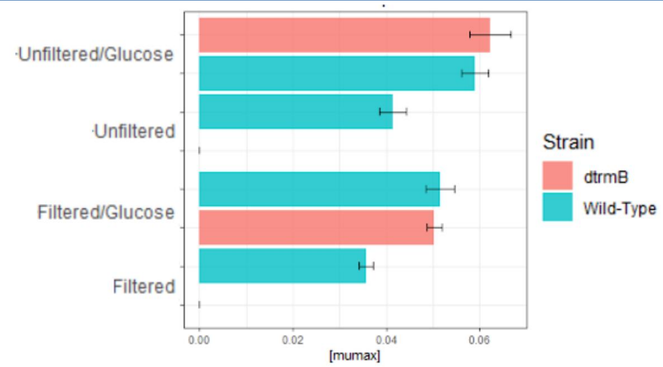

Figure 4. Maximum growth rate (μ_{max}) of Δ*trmB* and wild-type in different conditions, including filtered and unfiltered media with the presence or absence of glucose. μ_{max} is non-parametrically estimated. Error bars produced from two standard errors.

Discussion & Conclusion

Investigation into *Hbt. salinarum* shows that it is dependent on glucose metabolism for proper membrane formation. In *Hca. hispanica*, however, there is no significant difference in the morphology of the Δ*trmB* without glucose, which indicates that its membrane composition may not be dependent on the metabolism of glucose. In addition, there is a significant difference between the growth rate of the Δ*trmB* with and without glucose in its media. Significance was determined through a Student's t-test. We can conclude that the growth of the Δ*trmB* acts in a similar fashion in *Hca. hispanica* when compared to *Hbt. salinarum*.

In future studies, it would be helpful to determine the exact role that glucose plays in *Haloarcula hispanica* and whether that includes other aspects aside from its involvement in growth regulation. Furthermore, we are interested in investigating the reason as to why the unfiltered media has a higher growth rate.

References & Acknowledgements

(1) Todor H, Dulmage K, Gillum N, Bain JR, Muehlbauer MJ, Schmid AK. 2014. A transcription factor links growth rate and metabolism in the hypersaline adapted archaeon *Halobacterium salinarum*.

(2) Zwietering MH, Jongenburger I, Rombouts FM, van 't Riet K. 1990. Modeling of the bacterial growth curve. Appl Environ Microbiol. 1990 Jun;56(6):1875-81.

We would like to thank Dr. Amy Schmid, Dr. Cynthia Darnell, and Rylee Hackley from the Schmid Lab at Duke University and Katie Jacobs, Laavanya Sankaranarayanan, and Chelsea Shoben from the Duke Genetics Program as well as Dr. Amy Sheck, Dr. Michael Bruno, and Dr. Darrell Spells from NCSSM.