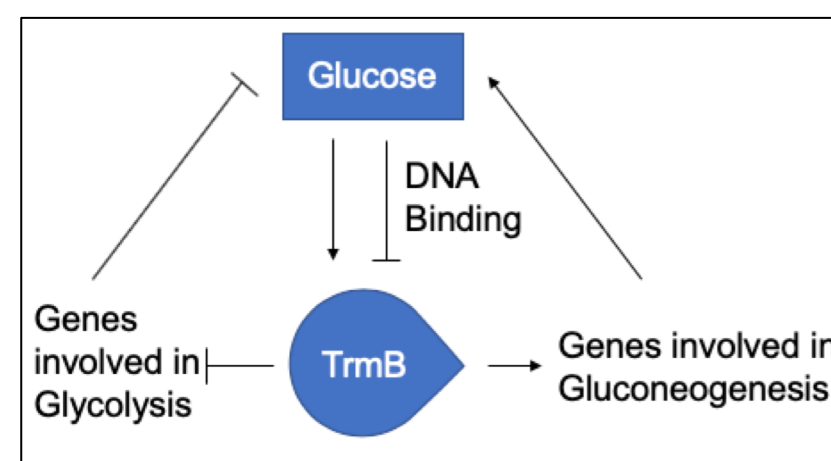


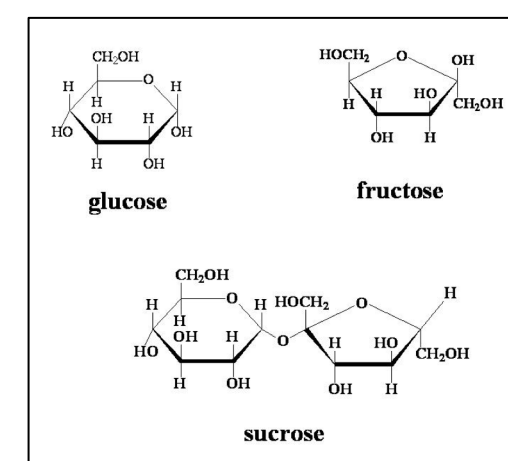
Introduction

Recently, scientists discovered that archaeobacteria were an entirely different domain from the traditional bacteria and eukarya classifications. The organisms have been observed as extremophiles. Differences in the RNA offer evidence that archaeobacteria diverged from bacteria and eukaryotes. In this experiment, the model organism was *Haloarcula hispanica*, which habitats an extremely salty environment.

TrmB is a transcription factor that is involved in metabolism throughout the archaea domain, and the regulation of sugars such as glucose. As a transcription factor, TrmB binds to the promoters of genes, repressing some promoters while activating others. In *Halobacterium salinarum*, TrmB regulates genes coding for enzymes involved in the metabolism of glucose, but since glucose cannot be catabolized, the role of TrmB and the regulation of metabolism in response to glucose was unknown. Glucose was found to affect cell shape and morphology through glycosylation.



Involvement of TrmB in regulation of metabolic pathways



Molecular structure of sugars used

Prior to performing this experiment, we knew that *Haloarcula hispanica*, unlike *Haloarcula salinarum*, is able to metabolize glucose. Since sucrose is a disaccharide composed of glucose and fructose, we hypothesize that ΔtrmB *Hca. hispanica* is able to grow similarly to the wild type *Hca. hispanica* in the presence of sucrose at 0.1% and 0.2% and potentially in the presence of fructose at 0.1%.

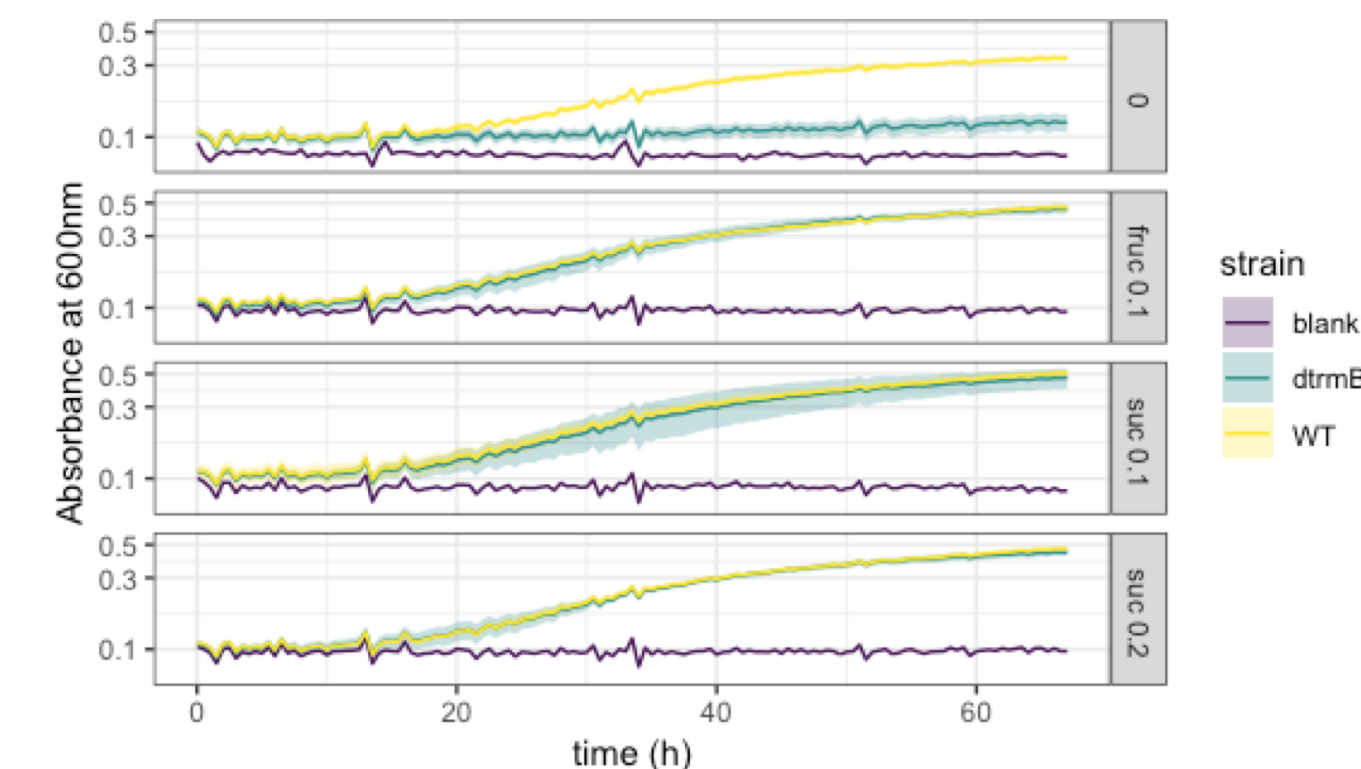
Methods

Each well was filled with one of 4 different media conditions, 4 biological replicates, 2 technical replicates and either wild-type or ΔtrmB strains of the *Haloarcula hispanica*. The media conditions tested were no sugar, 0.1% sucrose, 0.2% sucrose, and 0.1% fructose. The well plate was then incubated using a Bioscreen machine at 37° on the “fast” shaking setting, and optical density measurements were taken every 30 minutes between t=0 and t=67 hours.

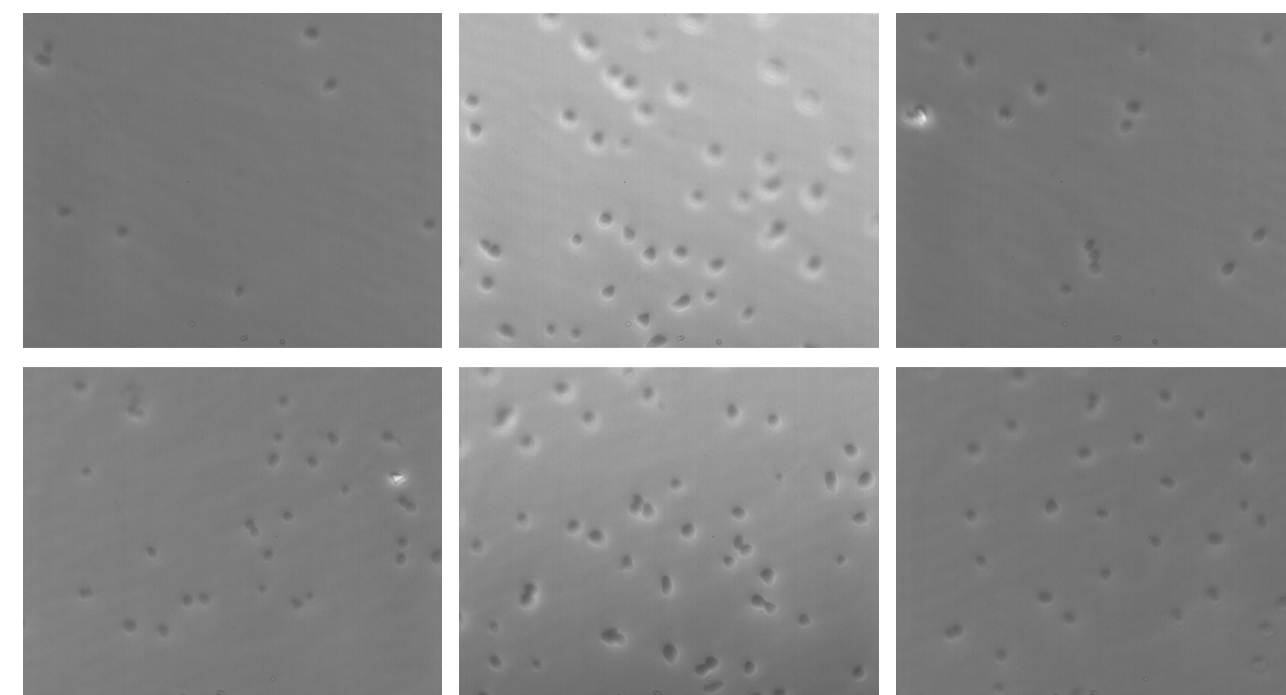
The optical density was the method of quantifying bacterial growth; as the optical density increases, so does the number of bacteria. Finally, the data collected were graphed and analyzed using RStudio, where several statistical tests were performed and models were fitted to the growth curves. In order to quantify the morphological differences across our *Hca. hispanica* cells, the ImageJ software was used to fit an ellipse to each cell and an average length:width ratio was calculated.

Results

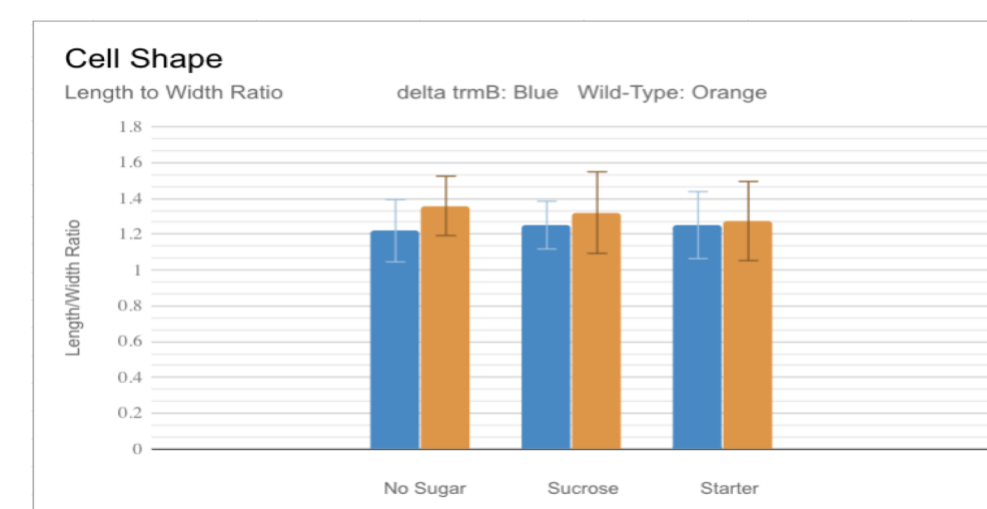
The addition of sucrose and fructose was able to rescue the growth defect in the ΔtrmB *Haloarcula hispanica*. Below are growth curves of each strain of *Hca. hispanica* separated by the condition.



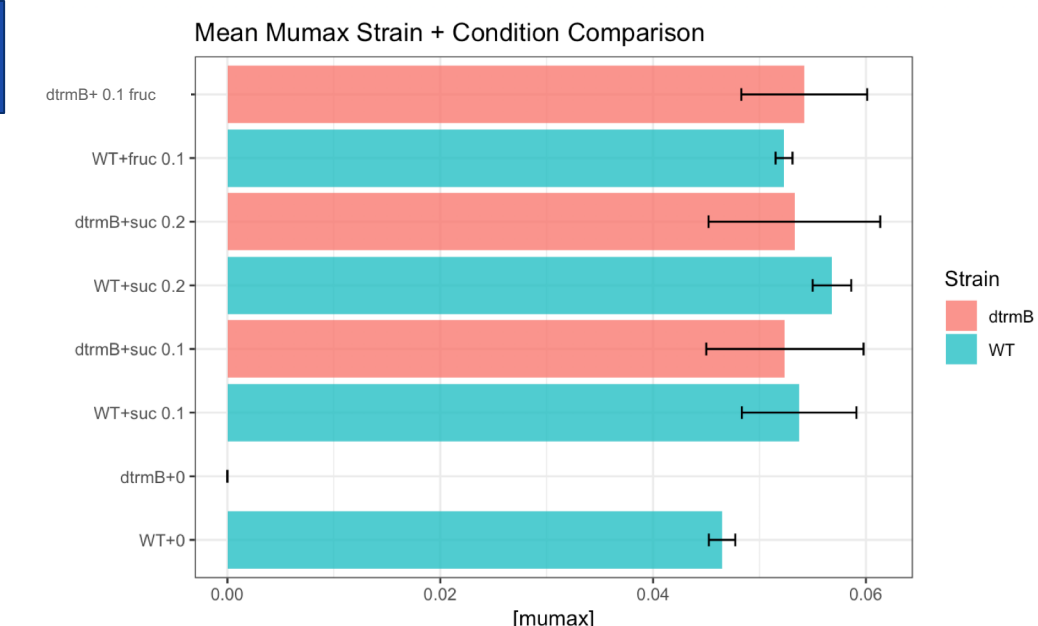
Wild type *Haloarcula hispanica* and ΔtrmB *Haloarcula. hispanica* are both morphologically round, independent of condition.



Above: A series of microscopy photographs captured at 100x resolution showing the morphology of ΔtrmB *Hca. hispanica* (top) and the wild type (bottom) with no sugar, the starter culture, and sucrose respectively.



The maximum growth rate for ΔtrmB *Haloarcula hispanica* with the addition of sucrose and fructose was statistically significantly higher as compared to the negative control. However, the maximum growth rate for ΔtrmB *Hca. hispanica* with the addition of sucrose and fructose as compared with that for wild type *Hca. hispanica* was not statistically significantly different.



Left: Bar graph of ΔtrmB *Hca. hispanica*'s average maximum growth rate compared to the wild type's under different conditions.

Discussion and Conclusion

- Growth rates of different sugars could be compared to that of glucose.
- The unfiltered media could have caused errors in the growth curve. The media used had peculiar clumps of salt that the haloarchaea was able to grow upon, which could have affected the OD measurements and impacted the growth rates of the haloarchaea.
- Lower sucrose concentrations should be used in the media, as the 0.1 and 0.2 concentration media yielded similar growth curves.
- Bioscreen incubators should run for longer, as we were not able to see the carrying capacity on the growth curve.
- Other sugars should be tested to see the different metabolic pathways available to the *Hca. hispanica*.
- Determine if *Hca. hispanica* uses glucose and other sugars for functions other than catabolizing, similar to how *Hbt. salinarum* uses glucose in its S-layer.

Both the ΔtrmB and wild type strains of *Hca. hispanica* can grow in media containing sucrose and fructose without a significant change in growth rates. The maximum growth rate of the ΔtrmB and the wild type strains are only significantly different when no sugar is present. This implies that the *Hca. hispanica* can cleave bonds between monomers in sucrose and metabolize fructose. The growth curve of *Hca. hispanica* can be modeled using a logistic and Gompertz growth curve. The wild-type and ΔtrmB strains of *Hca. hispanica* are morphologically round, providing no significant data on S-layer changes in ΔtrmB *Hca. hispanica*.

References and Acknowledgements

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