trmB Gene Expression in *Haloferax volcanii* in the Presence of Different Carbon Sources

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North Carolina School of Science and Mathematics - Courtesy of Schmid Lab at Duke University

Introduction

- A trmB deletion mutant strain, *ΔtrmB*, appears to be complemented by glucose in *H. salinarum*. Complementation means the mutation is no longer expressed (Todor, et al.).
- trmB is a transcription factor protein that activates other genes to assist in gluconeogenesis (the derivation of glucose). This glucose is used to create S-layer cell surface proteins, which *H. salinarum* and *H. volcanii* need to grow
- It is unclear if H. volcanii and H. salinarum use carbon sources and trmB in a similar way (Todor, et al.)

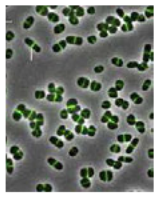

Figure 1.1: *Haloferax volcanii is a halophilic archaea with a similar genome to previously studied Halobacterium salinarum.*

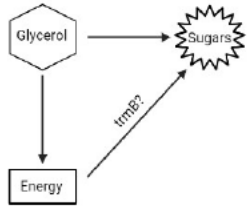

Figure 1.2: *It is unclear exactly what role trmB plays in the gluconeogenesis process of* H. volcanii *(BioRender)*

- Determine the effects of different carbon sources on *ΔtrmB* 's ability to generate its own glucose through gluconeogenesis
- **It is hypothesized that in the presence of any carbon source, *ΔtrmB* would exhibit growth similar to wild-type**.
- The optical density of cell cultures is measured to model their growth in the presence of these carbon sources.
- *ΔtrmB* exhibited the highest level of growth with glucose. Galactose and glycerol were close to the wild-type levels of growth. Xylose had a considerable population increase before tapering off and exhibiting similar patterns as no carbon.

Methods

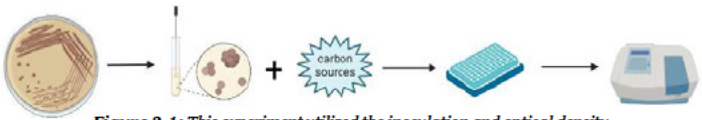

Figure 2.1: *This experiment utilized the inoculation and optical density measurement method used in the Todor, et al. experiment. (BioRender)*

Figure 2.2: *Haloferax volcanii after being spread into single cultures.*

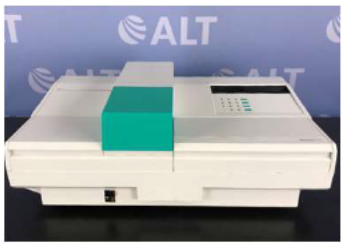

Figure 2.3: *The bioscreen machine used to collect OD values (Designs)*

Results

- In the presence of galactose and glycerol, *ΔtrmB* grows slightly less than WT, but still with relative success.
- Glucose complemented *ΔtrmB* the best, leading to growth rates higher than the WT sample
- In the presence of xylose, *ΔtrmB* grows less than the wild-type, although xylose's initial growth rate was comparable
- Both strains grew poorly in the absence of carbon

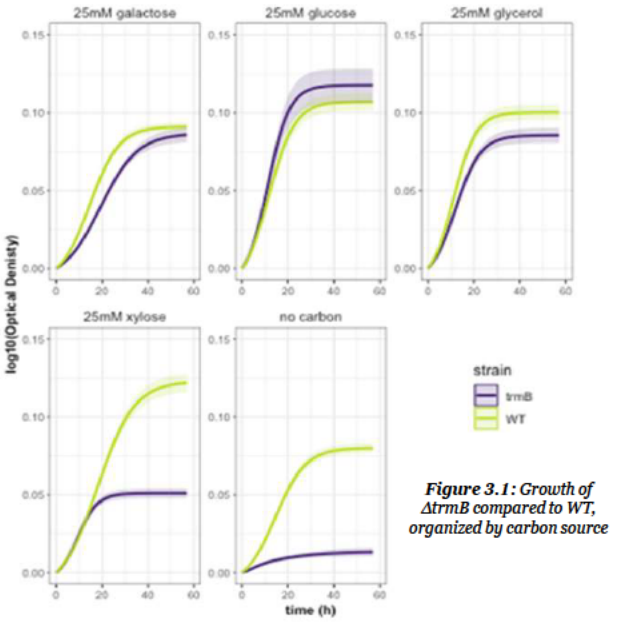

Figure 3.1: *Growth of ΔtrmB compared to WT, organized by carbon source*

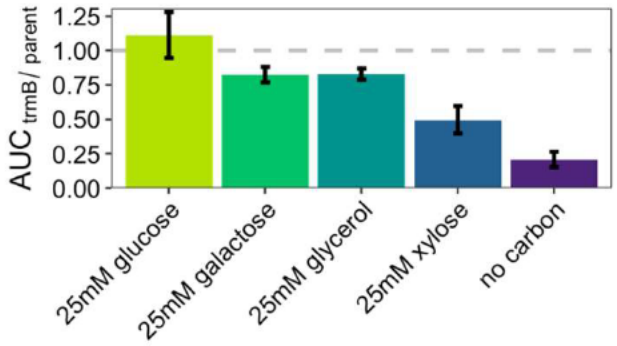

Figure 3.2: *Ratio between the growth of* ΔtrmB *and wild-type with indication of standard deviation.*

Conclusion

- **In the presence of glucose, galactose, and glycerol, the *ΔtrmB* mutation was complemented.**
- Due to *ΔtrmB* 's gluconeogenesis inhibition, when the samples were grown with no carbon, *ΔtrmB* grew less than the wild-type.`
- With no carbon, the wild-type's only source of glucose is produced through gluconeogenesis. *ΔtrmB* could not produce any supplemental glucose, which is why it barely grew.

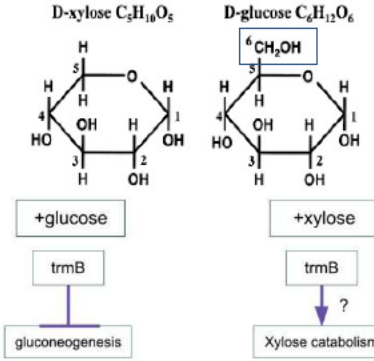

Figure 4.1: *Glucose is a more complex compound than xylose, so xylose cannot be derived from glucose. (Jackson)*

Figure 4.2: *The xylose data suggests trmB activates the XacR gene and is needed to activate xylose metabolism.*

- Further experimentation should be done to discover the differences in catabolizing xylose versus other carbon sources.
  - *ΔtrmB's* effect on S-layer growth with different carbon sources
  - *ΔtrmB's* effect on cell morphology
- *ΔtrmB* affects metabolic processes of one of the oldest living organisms, hinting toward how metabolic pathways evolved.
- Our results support further research into archaeal metabolic pathways.

References

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