Effect of the tbsP Gene on *Haloferax volcanii* Growth and Morphology

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Introduction

Haloferax volcanii, a group of halophile archaea, has been suggested to use the TrmB transcription factor involved in the metabolism of carbohydrate precursors of S-layer glycosylation, shape, and growth[1]. The wildtype *H. volcanii* has a rod-like shape. A recently discovered mutation introduces the tbsP gene, which we hypothesize is a suppressor for the Δ*trmB* mutant.

Goal: Identify the effect of the tbsP gene on *H.volcanii* growth and morphology.

Four different mutants of the same strain (Δ*pyrE2*/wildtype, Δ*trmB*, Δ*tbsP*, and Δ*trmB* + Δ*tbsP* (Δ*BP*)) of *H. volcanii* were tested in minimal media with or without glucose (+/-).

Overall, our evidence suggests that the tbsP gene is capable of suppressing the round-shape phenotype of the Δ*trmB* mutant in the absence of glucose, thereby recovering the wildtype phenotype of a rod-like shape.

Image Microscopy of Mutant Strains

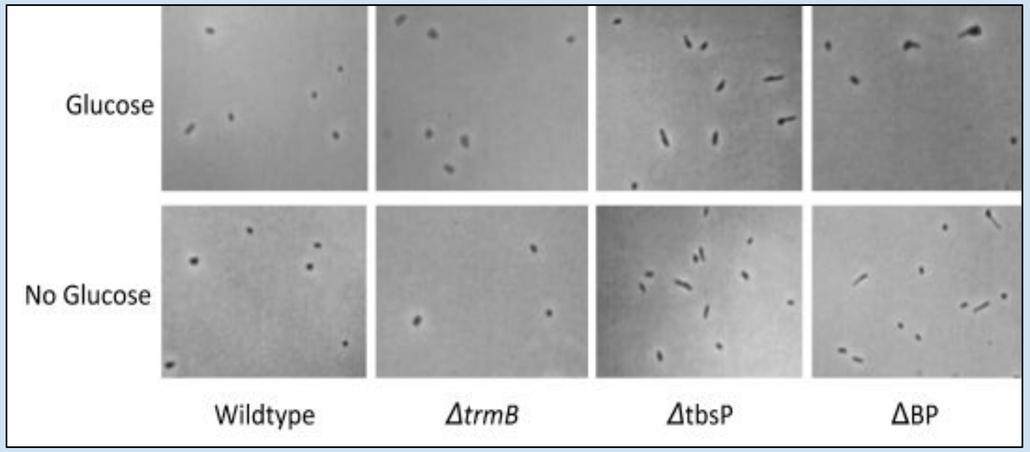
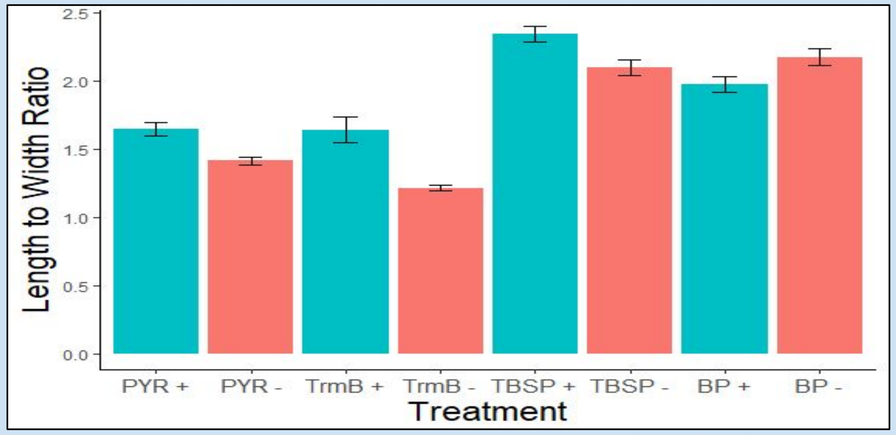


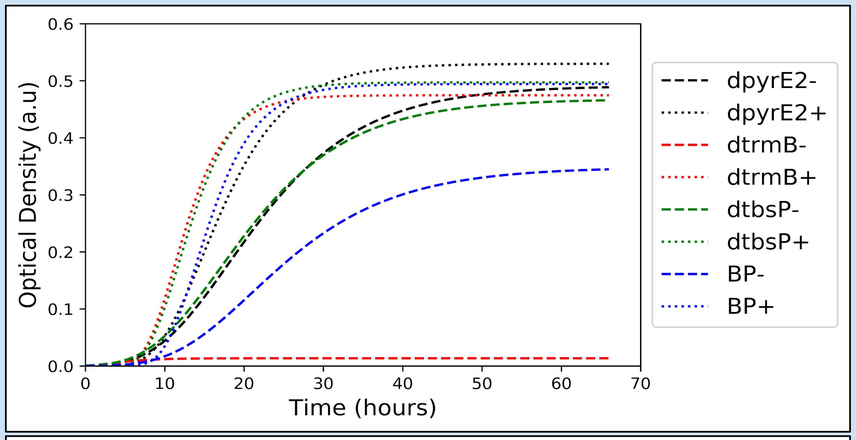
Image microscopy of the mutants shows the Δ*trmB* mutant without glucose has a rounder shape in comparison to the wildtype. The Δ*tbsP* and Δ*BP* mutant both have more rod-like shapes.

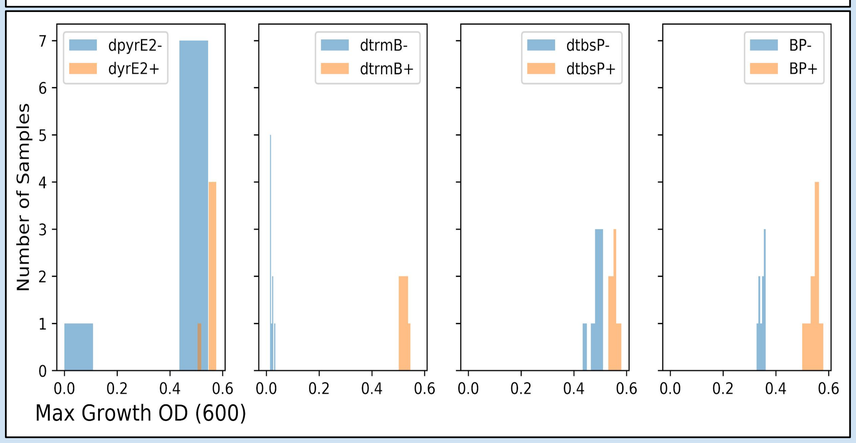
Quantification of Mutant Morphology



The average length to width ratio of cells for each mutant is compared. Δ*BP* appears to have a larger length to width ratio than Δ*trmB*, suggesting Δ*tbsP* is responsible for a longer rod-like shape. Δ*tbsP* appears to develop an even longer rod-like shape than its wild type, Δ*pyr*.

Variation in Growth Curves and Max Growth





A BioScreen was used to record the optical density (OD) of each strain for ~66 hours. The OD data was used to model a Gompertz growth curve of each mutant[2]. By assessing the maximum growth of each mutant with and without glucose, we found that the ΔtrmB mutant did not grow in the absence of glucose whereas the ΔBP strain grew regardless of the presence of glucose. This supports our hypothesis that the tbsP gene recovers the wildtype phenotype in the Δ*trmB* mutant.

Future Work

Further research is necessary to determine how exactly tbsP acts to suppress the Δ*trmb* mutation and if a glucose substitute is used in growth and morphology. Moreover, we will further analyze *H. volcanii* in order to understand the possible benefits of having a rod-like shape.

References

[1] Todor, H., Dulmage, K., Gillum, N., Bain, J. R., Muehlbauer, M. J. and Schmid, A. K. (2014), A transcription factor links growth rate and metabolism in the hypersaline adapted archaeon *Halobacterium salinarum*. Molecular Microbiology, 93: 1172–1182. doi:10.1111/mmi.12726

[2] Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & van 't Riet, K. (1990). Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology*, *56*(6), 1875–1881.

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