Growth Characterization of *env7Δ* under Hyperosmotic conditions

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ABSTRACT: Defects in lysosomal functioning and protein trafficking are associated with lysosomal storage diseases and neurodegeneration. *Saccharomyces cerevisiae*, the model organism used since vacuoles are functionally analogous to mammalian lysosomes. The gene *ENV7*, encodes the active protein kinase Env7, that plays a role in the negative regulation of vacuole membrane fusion in hyperosmotic condition and vacuole biogenesis. *ENV7* affects cell growth rate under hyperosmotic stress. *ENV7* is tested in high salt conditions, determining the growth rate of *ENV7* knockout (*env7Δ)* under hyperosmotic conditions compared to WT growth. In preliminary results, cells grown in rich yeast (YPD) media is over 2x higher for WT and *env7Δ* than under hyperosmolarity (1.5M NaCl + YPD). Furthermore, in both medium WT cells grow faster than *env7Δ* cells. Additionally, similar growth assays will be performed in synthetic minimal (SM) media to explore future studies on plasmid encoded ENV7 mutant species.

INTRODUCTION:

- Neurodegenerative and protein trafficking diseases like Alzheimer, Parkinson, Huntington are linked to defective lysosomal fusion dynamics.
- Research in Gharakhanian lab aims to understand the cellular machinery involved in trafficking cargo to the lysosome as well as it's correct functioning. Using the model organism *S.cerevisiae.*
- Under hyperosmotic stress, the vacuole fragments to maintain osmotic balance. *ENV7* (an ortholog of the human gene *STK16*) negatively regulates vacuolar fusion in cells during hyperosmotic stress.
- *ENV7* affects cell growth rate under hyperosmotic stress.

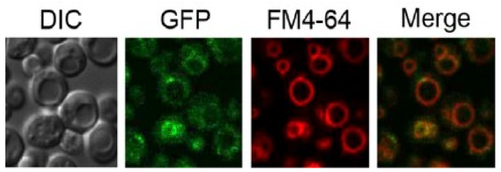


Fig 1 DIC and confocal microscopy showing wild-type yeast cells, confirming the localization of Env7-GFP tagged to the vacuole membrane.

METHODS:

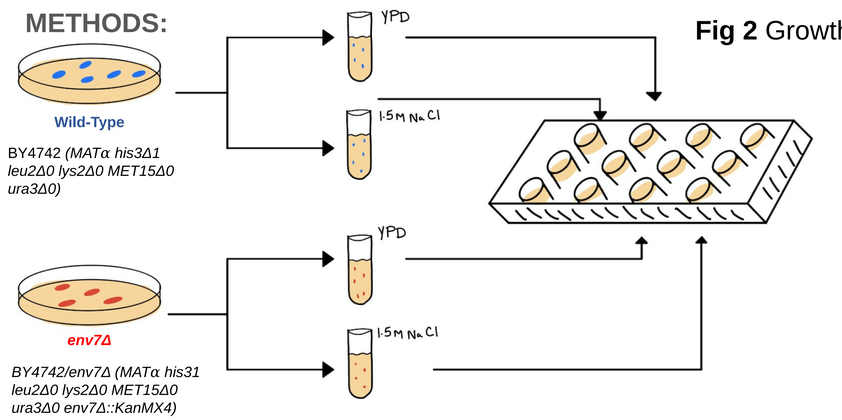
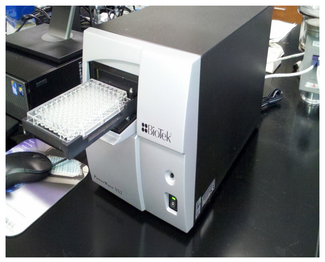
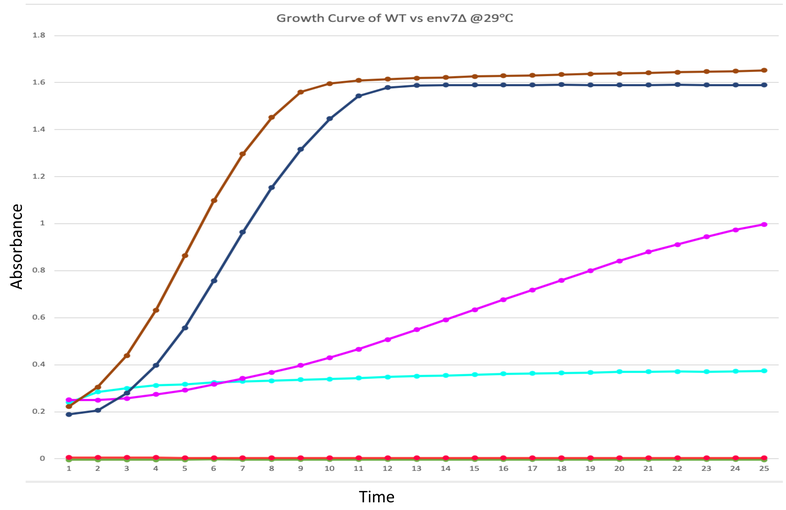


Fig 2 Growth Assay



- Single colonies of *S. cerevisiae* are grown on yeast extract peptone dextrose (YPD) media.
- YPD or high salt YPD is inoculated with single colonies to grow over night.
- Cells are diluted to 0.2OD (early log) growth phase in 96-well plates with or without 1.5M NaCl, ran in triplicates
- Cell density was quantitated in time using multiplate reader set at 600nm wavelength to determine concentration of cells in liquid

RESULTS:



- Brown: WT in YPD
- Blue: *env7Δ* in YPD
- Pink: WT in 1.5M NaCl YPD
- Teal: *env7Δ* in 1.5M NaCl YPD
- Red: YPD Blank
- Green: 1.5M NaCl Blank

Fig 3 Average of triplicate results from each condition. 24-hour growth rate of strains in specified media.

In preliminary results both strains grow over 2x greater in YPD than in 1.5M NaCl YPD. Furthermore, in stress conditions WT grows better than *env7Δ.*

CONCLUSION & FUTURE WORK:

- In high salt conditions cells grow significantly less regardless of *ENV7.*
- *ENV7* positively affects cell growth under hyperosmotic stress.
- In future, repeat same experiment for reproduceable results. Then perform the same experimental approach in synthetic minimal (SM) media for future plasmid-based selection experiments.

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