

SPATIOTEMPORAL METABOLIC HETEROGENEITY IN CLONAL YEAST COMMUNITIES

Dr. Sriram Varahan Lab, CSIR-CCMB
(unpublished data)



Adishree M

AIM

To develop molecular tools to characterize metabolic processes orchestrating fungal morphogenesis.

INTRODUCTION

- **Morphogenesis** is the biological process by which organisms develop their shape, form, and overall structure during their life cycle.
- It involves a series of tightly regulated and coordinated events that lead to the creation of the body plan and the arrangement of cells into specific tissues, organs, and structures.
- Fungi, being a diverse group of organisms, exhibit various morphological forms and can switch between these forms based on environmental cues.
- **Pseudohyphal differentiation** is a process observed in certain fungi, particularly in species of yeasts, where individual yeast cells form elongated chains or filaments that resemble hyphae but are structurally different. Understanding fungal morphogenesis in the context of pseudohyphal differentiation involves specific characteristics and regulatory mechanisms:
 - i) Pseudohyphal growth is induced by specific environmental cues, such as nutrient limitation (like nitrogen starvation), low nitrogen-to-carbon ratio, or other stresses. These conditions trigger signaling pathways that promote pseudohyphal differentiation. [1]
 - ii) Pseudohyphal differentiation is regulated by complex signaling pathways. In *Saccharomyces cerevisiae*, for instance, the mitogen-activated protein kinase (MAPK) pathway, particularly the **MAP kinase cascade (Ste20-Ste11-Ste7-Fus3)**, plays a crucial role in inducing pseudohyphal growth in response to nutrient limitations. [2]
 - iii) Pseudohyphal cells display increased adhesion properties compared to individual yeast cells. This enhanced adhesion is vital for forming multicellular structures and may contribute to improved nutrient uptake and environmental survival.
 - iv) Role in Virulence and Pathogenesis: In certain pathogenic fungi, pseudohyphal growth can be associated with increased virulence. For instance, *Candida albicans*, a pathogenic yeast, can transition to a pseudohyphal form, aiding in tissue invasion and immune evasion during infection.[3]
 - v) Evolutionary Significance: Pseudohyphal differentiation might represent an adaptive strategy for fungi to cope with changing environmental conditions. This trait allows the formation of multicellular structures while maintaining the advantages of a unicellular lifestyle.

OBJECTIVES

1. To determine the metabolic state of pseudohyphal cells.
2. To test whether spatiotemporal metabolic heterogeneity influences pseudohyphal differentiation.

RATIONALE

Preliminary studies showed nitrogen limitation and the presence of fermentable carbon sources to be important for pseudohyphal development[1]. In this project, I aim to determine the spatio-temporal activity/expression of glycolysis when *S. cerevisiae* undergoes pseudohyphal differentiation. This, in turn, will allow us to understand how these metabolic pathways drive these morphogenetic switching events.

GENES		About	Expected observations
<i>TKL1</i>	TransKetoLase	<ul style="list-style-type: none">• Key enzyme involved in the pentose phosphate pathway.• creates a reversible link between two main metabolic pathways, the pentose phosphate pathway, and glycolysis.	<u>Tkl1-mCherry reporter assay:</u> <ul style="list-style-type: none">• In the presence of glucose, Tkl expression goes up.• Pseudohyphal cells should show significantly higher fluorescence than central cells, in the presence of glucose.
<i>ICL1</i>	IsoCitrate Lyase	<ul style="list-style-type: none">• Involved in glyoxylate cycle.• ICL1 expression is induced by growth on ethanol and repressed by growth on glucose.	<u>Icl1-mCherry reporter assay:</u> <ul style="list-style-type: none">• Icl expression is repressed in the presence of glucose.• Pseudohyphal cells shouldn't show any fluorescence activity in presence of glucose.

CDC19 (PYK1)	Pyruvate Kinase	<ul style="list-style-type: none"> converts phosphoenolpyruvate to pyruvate. Rate limiting glycolytic enzyme. substrate for PKA Transcriptionally regulated by the presence of glucose. It can be tagged c-terminally. 	<u>Cdc19-GFP reporter assay:</u> <ul style="list-style-type: none"> Cdc19 expression goes up in the presence of glucose. Pseudohyphal cells should show significantly higher fluorescence activity than central cells, in the presence of glucose.
PGK1	3-PhosphoGlycerate Kinase	<ul style="list-style-type: none"> Transcriptionally regulated by the presence of glucose. High expression in glucose-excess conditions. It can be tagged c-terminally. 	<u>Pgk1-GFP reporter assay:</u> <ul style="list-style-type: none"> Pgk1 expression goes up in the presence of glucose. Pseudohyphal cells should show significantly higher fluorescence activity than central cells, in the presence of glucose.
TDH3	Triose-phosphate DeHydrogenase	<ul style="list-style-type: none"> involved in glycolysis and gluconeogenesis. It can be tagged c-terminally. 	<u>Tdh3-GFP reporter assay:</u> <ul style="list-style-type: none"> Tdh3 expression goes up in the presence of glucose. Pseudohyphal cells should show significantly higher fluorescence activity than central cells, in the presence of glucose.

METHODOLOGY, RESULTS AND CONCLUSION

(I) EPITOPE TAGGING: To check the temporal expression of glycolytic enzymes using western blotting.

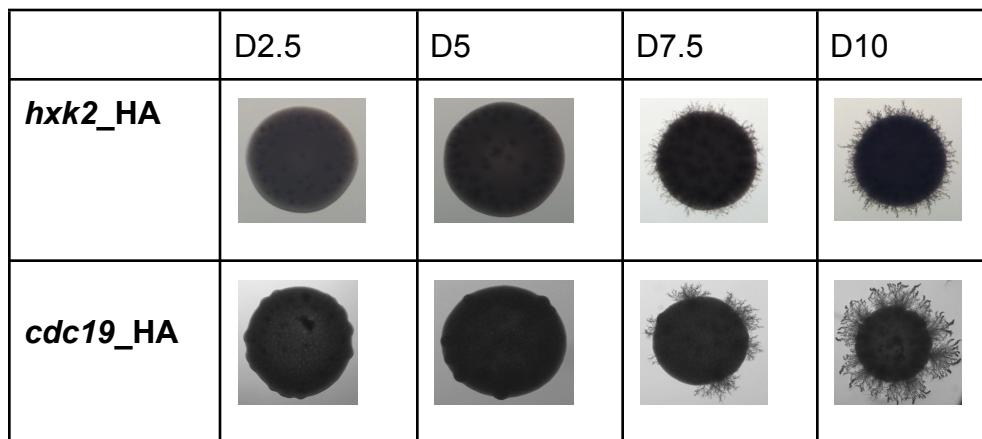
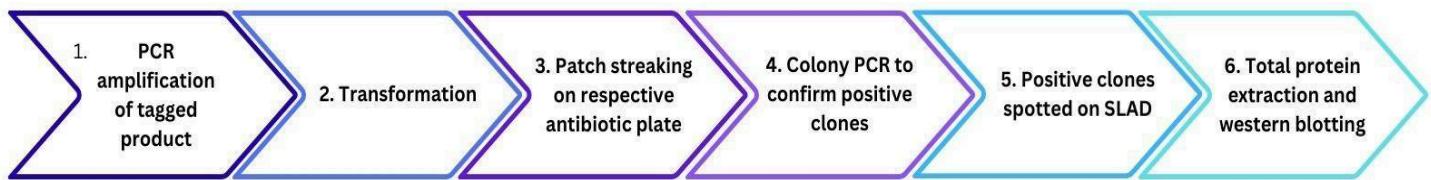


Figure: Pyruvate kinase (Cdc19) and Hexokinase (Hxk2) were epitope-tagged to determine the temporal expression of these glycolytic enzymes. Colonies that were spotted on synthetic low ammonium dextrose (SLAD) media were imaged.

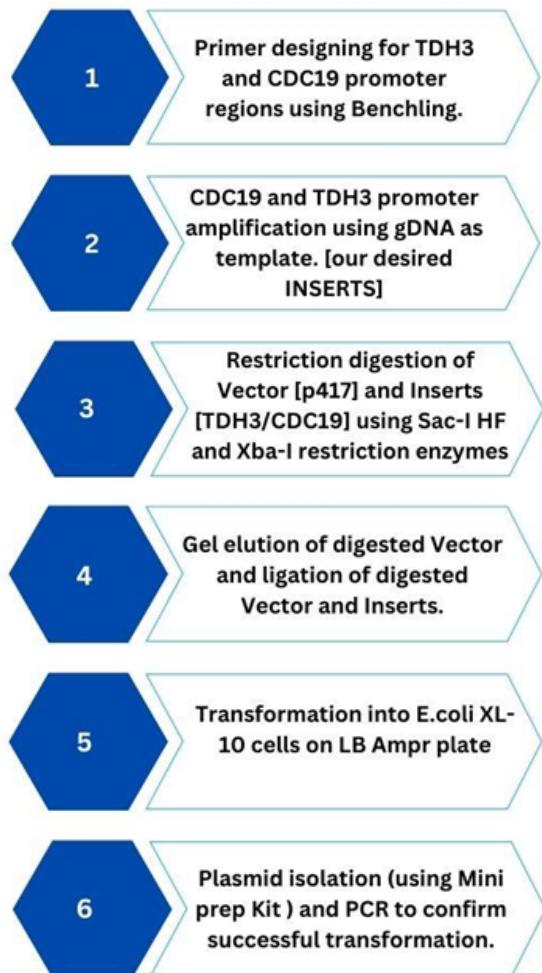


Figure: Hexokinase (Hxk2) and Pyruvate kinase (Cdc19) spotted on SLAD were isolated for western blotting.

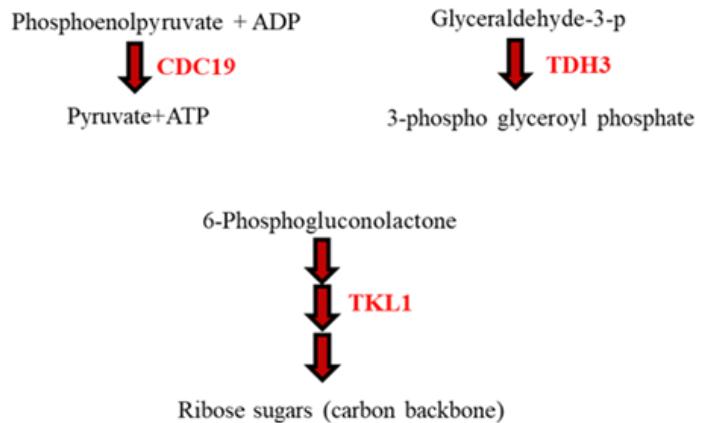
Results and Conclusion

While Hxk2 levels seem to decrease, levels of *cdc19* are uniform throughout the process of pseudohyphal differentiation.

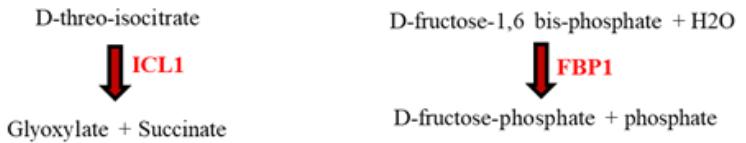
(II) PROMOTER-REPORTER ASSAY: To check the spatial activity of glycolysis and related pathways using fluorescence microscopy.

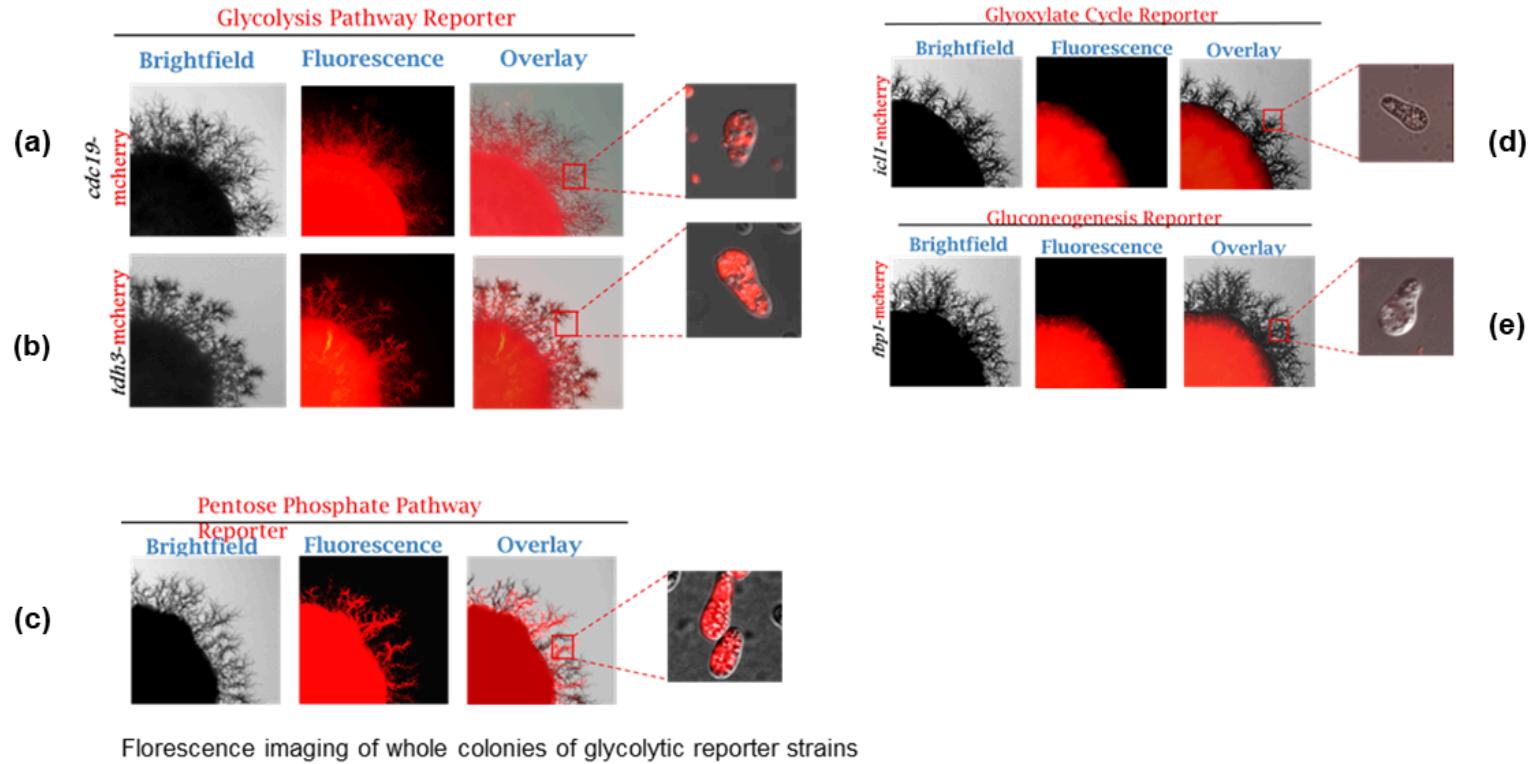


Glucose de-repressed genes



Glucose repressed genes





Fluorescence imaging of whole colonies of glycolytic reporter strains

- (a) Reporter strain where mCherry expression is under the control of pyruvate kinase (*cdc19*) promoter.
- (b) Reporter strain where mCherry expression is under the control of glyceraldehyde-3-phosphate dehydrogenase (*tdh3*) promoter.
- (c) Reporter strain where mCherry expression is under the control of transketolase (*tkl1*) promoter.
- (d) Reporter strain where mCherry expression is under the control of isocitrate lyase (*icl1*) promoter.
- (e) Reporter strain where mCherry expression is under the control of fructose bisphosphatase (*fbp1*) promoter.

Results and Conclusion

Expression of *tdh3* and *tkl1* is uniform throughout the process of pseudohyphal differentiation.
icl1 and *fbp1* expression seems to be highest on day 7.5

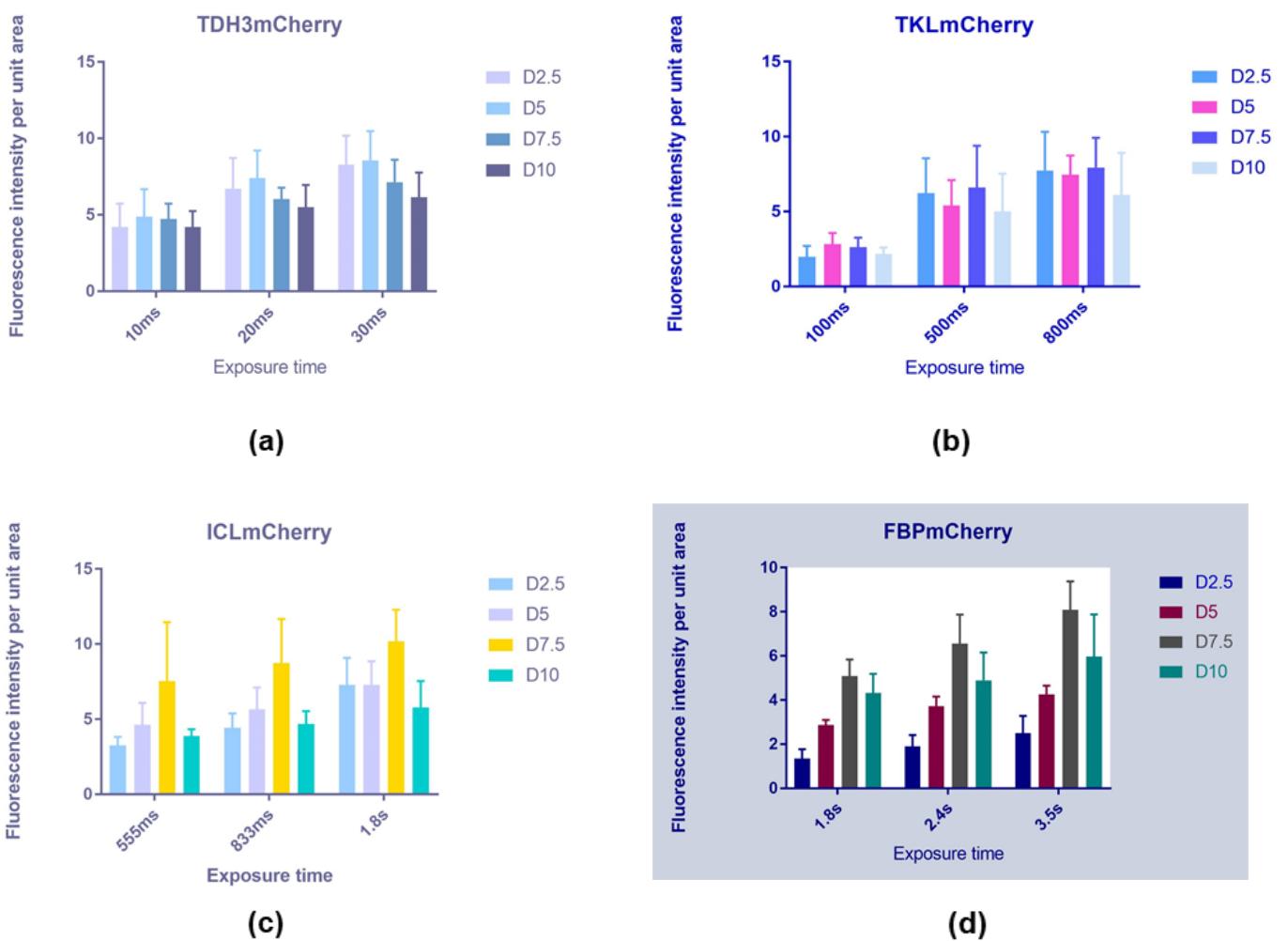


Figure: (whole colony) Fluorescence quantification of glycolytic reporter strains on different days and at different exposures. (a)TDH3mCherry, (b) TKLmCherry, (c) ICLmCherry, (d) FBPMCherry

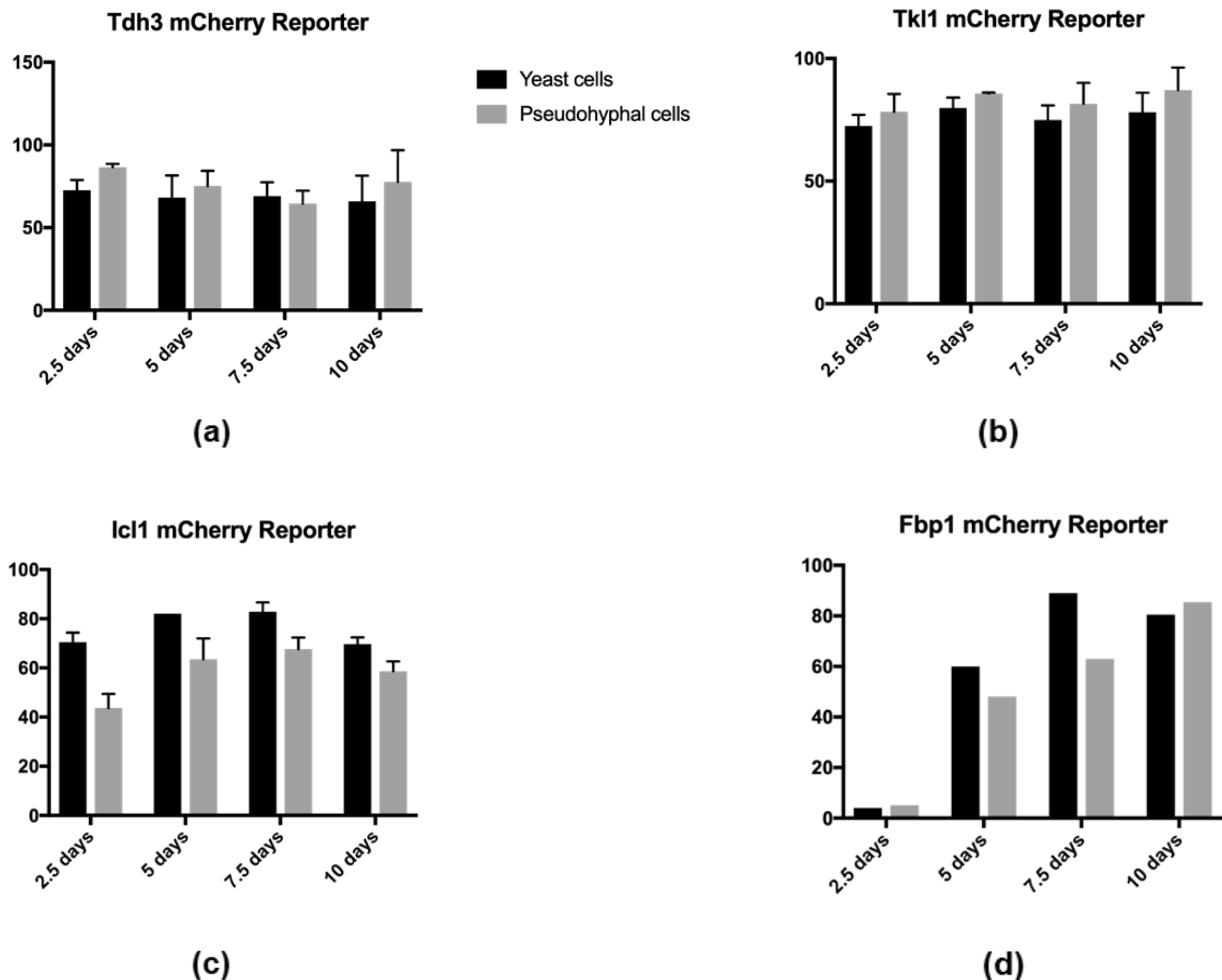


Figure: (Single-cell analysis) Percentage of fluorescent yeast and pseudohyphal cells in different reporter strains on different days. (a) *TDH3mCherry*, (b) *TKLmCherry*, (c) *ICLmCherry*, (d) *FBPmCherry*

(III) FLUORESCENCE TAGGING: To check the spatio-temporal expression of glycolytic enzymes using fluorescence imaging.

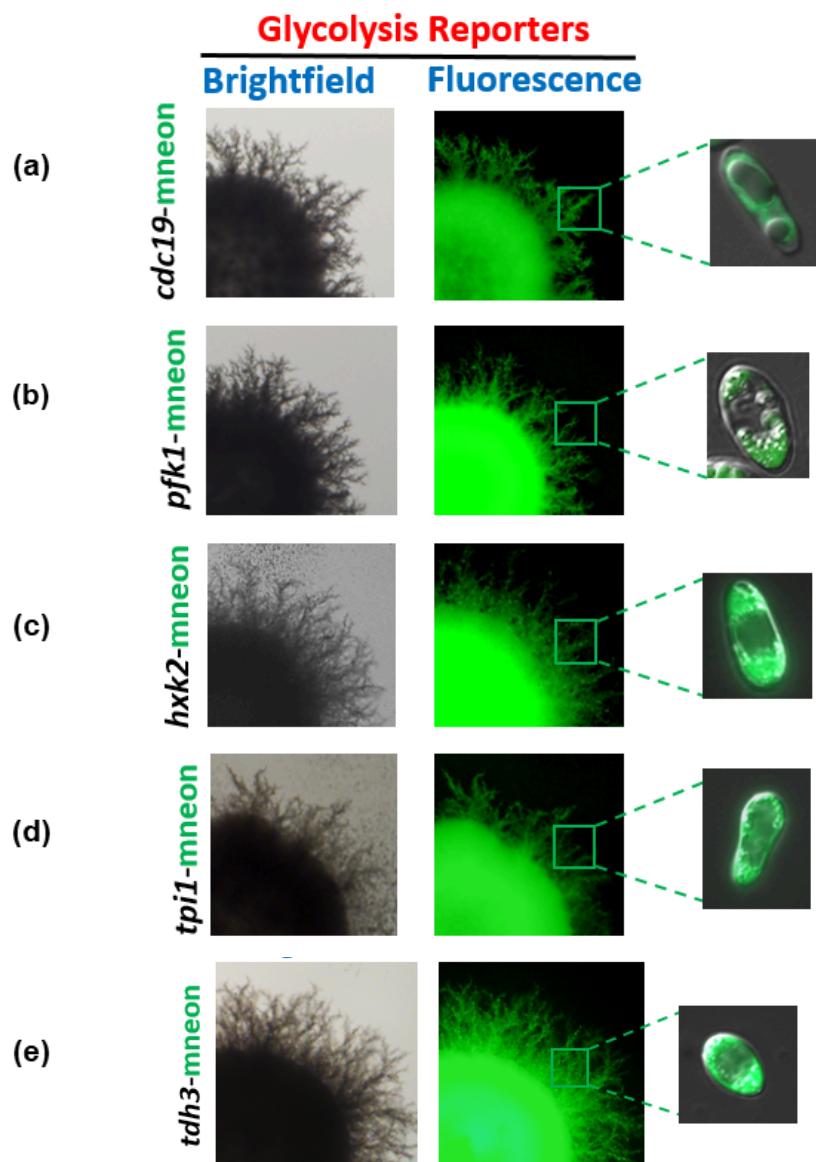
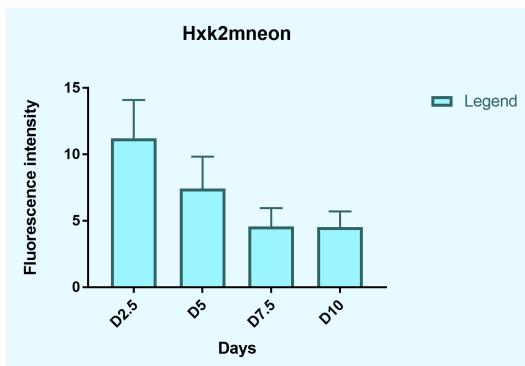
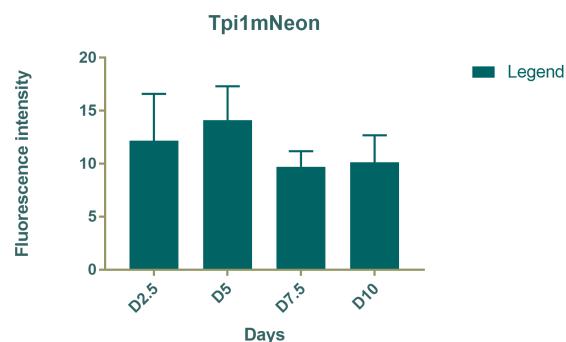


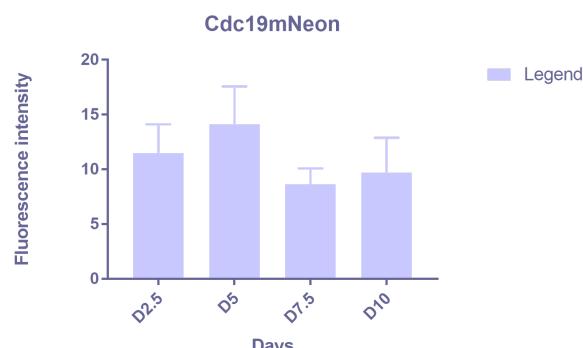
Figure: (a) Pyruvate kinase (Cdc19), (b) Phosphofructokinase (Pfk1), (c) Hexokinase (Hxp2), (d) Triosephosphate isomerase (Tpi1), and (e) Glyceraldehyde-3-phosphate dehydrogenase (Tdh3) were tagged with mNeon to determine the spatiotemporal expression of these glycolytic enzymes. Colonies that were spotted on SLAD were imaged on different days and at different exposures: single-cell imaging of the same was done on day 10 only.



(a)



(b)



(c)

Figure: Fluorescence quantification of mNeon tagged glycolytic enzymes: (a) Hexokinase (Hxk2), (b) Triosephosphate isomerase (Tpi1), (c) Pyruvate kinase (Cdc19), were tagged with mNeon to determine the spatiotemporal expression of these glycolytic enzymes. Colonies that were spotted on SLAD were imaged on different days and at different exposures: expression levels was checked by fluorescence quantification of whole colonies.

Results and Conclusion

From the whole colony imaging data, expression of glycolytic enzymes involved in later steps of glycolysis, like *Tpi1* and *Cdc19* seems to be higher in the periphery than ones involved in earlier steps of glycolysis, like *Hxk2*.

Single-cell imaging data is inconclusive as expression of glycolytic enzymes seems to be diffused all over the cells and not localized.

References

1. BROWN, C., HOUGH, J. *Elongation of Yeast Cells in Continuous Culture*. *Nature* 206, 676–678 (1965). <https://doi.org/10.1038/206676a0>
2. Cullen PJ, Sprague GF Jr. *The regulation of filamentous growth in yeast*. *Genetics*. 2012 Jan;190(1):23-49. doi: 10.1534/genetics.111.127456. PMID: 22219507; PMCID: PMC3249369.
3. Carlos J. Gimeno; Per O. Ljungdahl; Cora A. Styles; Gerald R. Fink (1992). *Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: Regulation by starvation and RAS*. , 68(6), 1077–1090. doi:10.1016/0092-8674(92)90079-r