DEVELOPMENT OF MOLECULAR TOOLS TO UNDERSTAND METABOLIC REGULATION OF FUNGAL MORPHOGENESIS

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ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to RICH-STEM program for providing me the platform to resume my career in science. My sincere thanks to Mrs. Aboli Ingle for her continuous support, motivation, and guidance.

I would like to extend my gratitude to my supervisor and mentor, Dr. Sriram Varahan for giving me the opportunity to work on this exciting and innovative project and for encouraging me to do my best. I thank my fellow lab mates Dhrumi Shah, Avanti Vairagkar, Nivedhitha R, Riya Bhattacharjee and Nikita Rewatkar for the stimulating discussions, for always lending a helping hand, and for all the fun we have had in the past months. I thank CSIR-CCMB for providing me the infrastructure and resources needed for my project.

Lastly, I would like to thank my friends and family for their endless love and support.

AIM

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

OBJECTIVES

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

INTRODUCTION

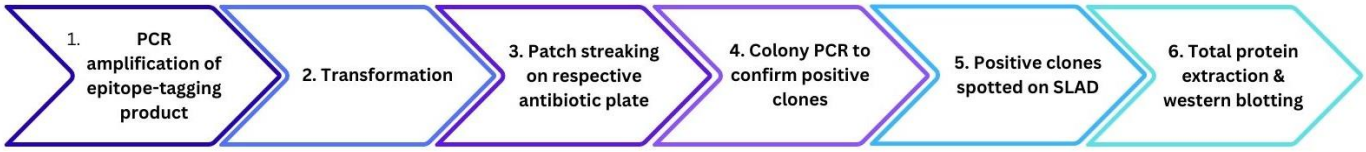
- Cells in an isogenic population are capable of showing visual, behavioral, and functional heterogeneity.
- Studying pseudohyphal differentiation in *Saccharomyces cerevisiae* can provide fundamental insights into understanding the general biological mechanisms that underlie the adaptation of microorganisms to different environments.

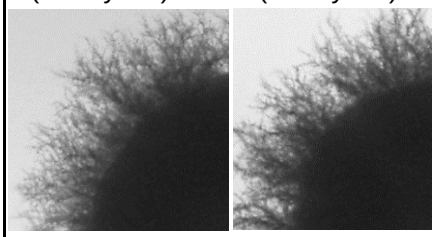
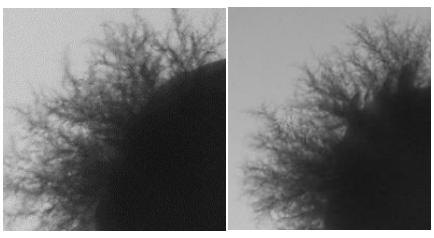
RATIONALE

Previous studies in the lab identified central carbon metabolic pathways like glycolysis and pentose phosphate pathway (PPP) to be critical for pseudohyphal differentiation. The aim of my project was 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.

METHODOLOGY, RESULTS & CONCLUSION

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

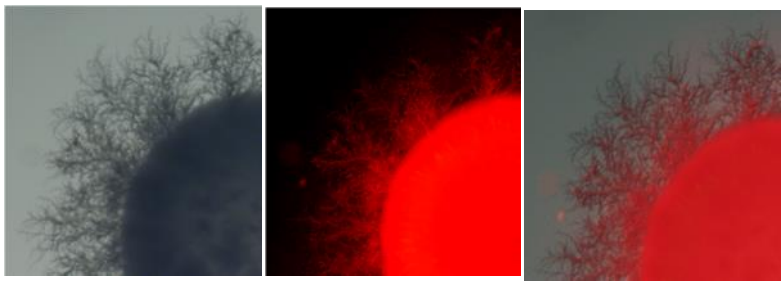


Strain: Cdc19-HA (Epitope tagged)	Hxk2-HA (Epitope tagged)	Pgi1-HA (Epitope tagged)
(colony – 1) (colony – 2)	(colony – 1) (colony – 2)	(colony – 1) (colony – 2)
		
[SLAD day 10 imaging]	[SLAD day 10 imaging]	[SLAD day 10 imaging]

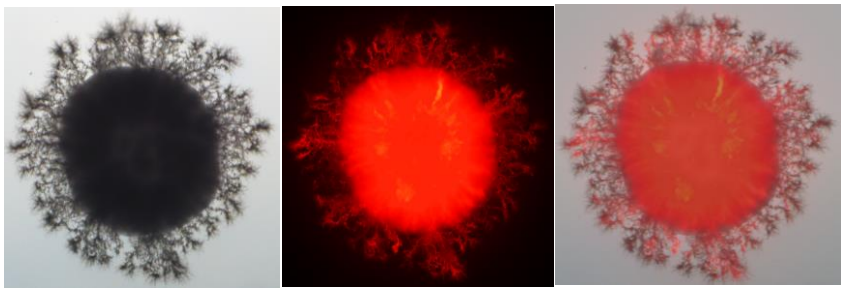
Pyruvate kinase (Cdc19), hexokinase (Hxk2) and phosphoglucose isomerase (Pgi1) were epitope tagged to determine the spatio-temporal expression of these glycolytic enzymes. Colonies that were spotted on synthetic low ammonium dextrose (SLAD) media were imaged and cells were isolated for western blotting.

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

1. Primer designing for t*dh3* and c*dc19* promoter regions using Benchling.
2. *tdh3* and c*dc19* promoter amplification using gDNA as template. [our desired INSERTS]
3. Restriction digestion of vector [p417] and inserts [t*dh3 and cdc19*] using Sac-I HF and Xba-I restriction enzymes
4. Gel elution of digested vector and ligation of digested vector and inserts.
5. Transformation into *E.coli* XL-10 cells on LB Amp plate
6. Plasmid isolation (using Mini prep Kit) and PCR to confirm successful transformation.



(a)

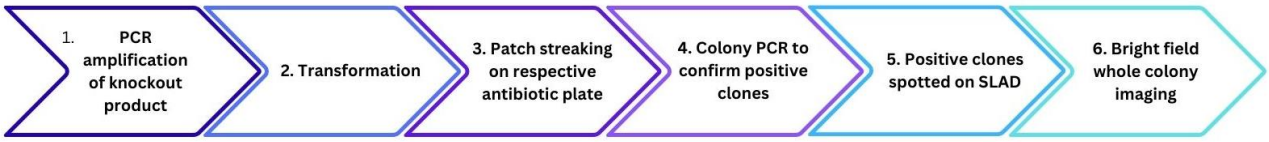


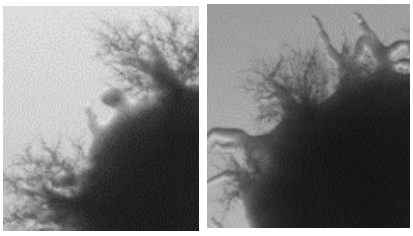
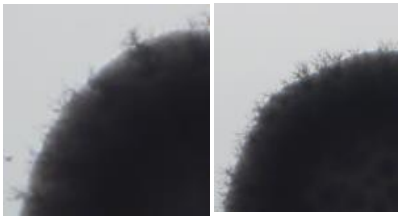
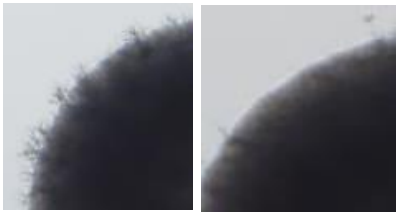
(b)

Florescence imaging of whole colonies of glycolytic reporter strains on day 14.
(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.

(III) **GENERATION OF KNOCKOUT STRAINS**

Previous studies in the field have shown that two strains namely, ∑1278b and CEN.PK undergo pseudohyphal differentiation under nitrogen-limiting conditions (1,2). Work from Dr. Sriram Varahan's lab (CSIR-CCMB) has shown that glycolysis is critical for driving pseudohyphal differentiation since disruption of genes that encode for glycolytic enzymes, alcohol dehydrogenase (*adh1*) and phosphofructokinase (*pfk1*) resulted in significant decrease in pseudohyphal differentiation in the ∑1278b diploid strain. In order to determine if glycolysis-dependent regulation of pseudohyphal differentiation is conserved across yeast strains, we generated knockout strains that lack genes encoding for the glycolytic enzymes, *adh1* and *pfk1* in the CEN.PK strain background using the following protocol.



CEN.PK (WT)	ΔΔ*adh1*	ΔΔ*pfk1*
(colony – 1) (colony – 2)	(colony – 1) (colony – 2)	(colony – 1) (colony – 2)
		
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Genes encoding glycolytic enzymes, alcohol dehydrogenase (*adh1*) and phosphofructokinase (*pfk1*) were deleted using homologous recombination and then spotted on SLAD medium along with CEN.PK (WT). They were imaged after 10 days. Disruption of *adh1* and *pfk1* in CEN.PK background results in significant reduction in pseudohyphal differentiation.

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