Understanding and Eliminating the Detrimental Effect of Thiamine Deficiency on the Oleaginous Yeast *Yarrowia lipolytica*

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Project Goals: To understand thiamine deficiency on cellular metabolism, elucidate genetic basis causing thiamine auxotrophy, and identify thiamine-responsive elements in *Yarrowia lipolytica*.

Abstract text. Thiamine is a vitamin that functions as a cofactor for key enzymes in carbon and energy metabolism for all living cells. While most plants, fungi and bacteria can synthesize thiamine de novo, the oleaginous yeast, *Yarrowia lipolytica*, cannot. In this study, we used proteomics together with physiological characterization to understand key metabolic processes influenced and regulated by thiamine availability and identified the genetic basis of thiamine auxotrophy in *Y. lipolytica*. Specifically, we found thiamine depletion results in decreased protein abundance of the lipid biosynthesis pathways and energy metabolism (i.e., ATP synthase), attributing to the negligible growth and poor sugar assimilation observed in our study. Using comparative genomics, we identified the missing 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate synthase (THI13) in the de novo thiamine biosynthesis of *Y. lipolytica*, and discovered an exceptional promoter, P3, that exhibits strong activation or tight repression by low and high thiamine concentrations, respectively. Capitalizing on the strength of our thiamine-regulated promoter (P3) to express the missing gene from *Saccharomyces cerevisiae* (scTHI13), we engineered the thiamine-prototrophic *Y. lipolytica*. By comparing this engineered strain to the wildtype, we unveiled the tight relationship linking thiamine availability to lipid biosynthesis and demonstrated enhanced lipid production with thiamine supplementation in the engineered thiamine-prototrophic *Y. lipolytica*.(1)

References

1. Walker C, Ryu S, Giannone RJ, Garcia S, Trinh CT. 2020. Understanding and Eliminating the Detrimental Effect of Thiamine Deficiency on the Oleaginous Yeast *Yarrowia lipolytica*. Applied and Environmental Microbiology 86:e02299-19.

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