48. Reprogramming acetyl-CoA metabolism for efficient production of lipid biofuels in Yarrowia lipolytica

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**Project Goals:** We focused on achieving a fundamental understanding of the metabolic pathways of the oleaginous yeast *Yarrowia lipolytica* and developing tools to characterize and engineer it. More specifically, we aimed to improve its fermentation characteristics towards the development of a cost-effective process which converts renewable resources to lipids for biodiesel production. The conversion yield and volumetric productivity on various carbon sources are the key metrics for optimization.

*Yarrowia lipolytica*, an oleaginous yeast, can naturally accumulate large quantity of neutral lipids using a variety of carbon sources. Previous metabolic engineering efforts working on the acyl-CoA related pathways (Tai M et al, Metabolic engineering, 2013) have resulted in efficient triacylglyceride producers by increasing the carbon flux towards malonyl-CoA and sequestering fatty acyl-CoAs in neutral lipids. This was achieved through the overexpression of native acetyl-CoA carboxylase (ACC) and diacylglycerol acyltransferase (DGA1) genes which may subject to post-transcriptional and post-translational regulations that complicate the metabolic process and limit the pathway efficiency. For example, the *S. cerevisiae* ACC has more than 20 phosphorylation sites that are subject to allosteric or kinase-related regulations (Li et al, Biotechnology and Bioengineering, 2014). To circumvent these limitations, we engineered alternative heterologous pathways that rewire and optimize the supply of acyl-CoA precursors to unleash the metabolic potential of oleaginous yeast. Specifically a prokaryotic four subunit acetyl-CoA carboxylase along with a plant-derived diacylglycerol acyltransferase have been functionally expressed and led to efficient synthesis of lipid biofuels.

Another major obstacle for efficient production of lipids in *Y. lipolytica* is pertinent to the unique nitrogen starvation conditions which distinctly separate the lipid accumulation phase from the cell growth phase, which is industrially undesirable due to the relatively low productivity and yield. We have engineered alternative cytosolic acetyl-CoA pathways that are less sensitive to intrinsic nitrogen sensing mechanism and ROS (reactive oxygen species) -scavenging pathways to alleviate the ATP citrate lyase (ACL) internal regulation. In this way, we could partially decouple nitrogen starvation and lipogenesis and move the lipid production from stationary phase to exponential phase. As a result, we were able to achieve very high level of oil content and lipid productivity by reprogramming the acetyl-CoA metabolism in *Y. lipolytica*. Since acetyl-CoA, malonyl-CoA and other acyl-CoAs are direct precursors for production of oleochemicals including fatty alcohols, fatty alkanes and fatty alkyl esters, the strategies reported in this work should be applicable to develop an yeast biorefinery platform that potentially upgrades low value carbons to high value commodity chemicals.