METABOLIC ENGINEERING OF YEAST FOR THE SYNTHESIS OF FATTY ACID AND POLYKETIDE-BASED CHEMICALS

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Polyketides and fatty acids are of critical importance as biorenewable chemical precursors, biofuels, and pharmaceuticals. Both are synthesized via complex polyketide or fatty acid synthases, with many using acetyl-CoA and malonyl-CoA as starter and extender units. We have engineered and combined multiple pathways in the yeast Saccharomyces cerevisiae for the production of these valuable compounds and to allow the synthesis of novel variants. We have combined enzyme engineering (of the pathway and synthase enzymes), extensive metabolic pathway engineering for increased cofactor and precursor pools, and cultivation strategies to substantially increase titers and yields of a variety of products, including 6-methylsalicylic acid (6-MSA), dihydromonocolin L (DML; precursor to lovastatin), fatty acids (FAs) of varying lengths, and triacetic acid lactone (TAL). S. cerevisiae was engineered for the high-level production of TAL by overexpression of native and variant Gerbera hybrida 2-pyrone synthase (2-PS), engineering of the yeast metabolic pathways, and implementation of various cultivation strategies. These interventions increased TAL titer from 0.07 g/L to 10.5 g/L and yield from <1% to 44% of theoretical yield. Recent work has modified mitochondrial transport mechanisms and implemented cofactor-based driving forces as methods to enhance polyketide synthesis. Fatty acids are also of interest as both biofuel and chemical precursors. We have introduced heterologous fatty acid synthases into S. cerevisiae to allow the synthesis of short/medium chain free fatty acids (C6C12), and have done extensive pathway engineering to increase the levels and secretion of these and long-chain free fatty acids (C16-C18) to the culture medium. Pathway engineering approaches have focused on increasing carbon flux from glucose into the fatty acid and neutral lipid forming pathways, and preventing degradation and re-activation of these fatty acids. A unique combination of gene knockouts and gene overexpression resulted in extracellular long chain FFAs at a titer of 2.2 g/L. Recent work has included enhancing resistance to C6, C8, and C10 fatty acid toxicity, novel approaches for medium chain fatty acid synthesis, and engineering of native yeast regulatory systems to increase synthesis of both polyketides and fatty acids. In the presentation, we will discuss the critical pathways engineered, and examine the synergy between successful strategies for the various fatty acid and polyketide products. We will also present our current research using novel applications of CRISPR/Cas9 to both rapidly select and combine pathway interventions to further increase synthesis and yield.