

83. Identifying biological pathway targets for lipid production in *Yarrowia lipolytica*

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Project Goals: Develop tools for cell-wide measurement of metabolites and lipids, which, along with genetic, light and electron microscopic and transcriptional analysis, will allow the construction of genome-scale metabolic models, as well as models of transcriptional regulation, that will guide the further metabolic engineering of *Yarrowia lipolytica*.

Yarrowia lipolytica is an oleaginous ascomycete yeast that is well known for its ability to accumulate large amounts of lipids. Despite a growing scientific literature focused on lipid production by *Yarrowia*, there remain significant knowledge gaps regarding the key biological processes involved. We are taking a multidisciplinary approach to identifying and characterizing the key pathways involved in *Yarrowia* lipid accumulation. A combination of mass spectrometry based metabolomics, light and electron microscopy and forward and reverse genetics is being applied to deepen our understanding of how this organism regulates and controls lipid production. We collected samples every 12 hours during a 5 day time course of *Yarrowia* growth under lipid producing conditions.

From these samples, we performed metabolomic analysis, super resolution fluorescence microscopy, helium ion microscopy and transmission electron microscopy. Our results indicate that cell wall biosynthesis may pull carbon flux away from lipid production making this an attractive target for future strain improvement efforts. In addition, we have developed a Nile Red-based genetic screen for *Yarrowia* mutant strains with altered lipid accumulation. We are currently characterizing these strains.

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