46. Determination of lipase gene products relevant to lipid mobilization in Yarrowia lipolytica and characterization of their respective knockout strains

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Project Goals: The purpose of this investigation is to improve lipid production in *Y. lipolytica* by knocking out lipase genes and preventing lipid mobilization. As *Y. lipolytica* possesses many genes annotated to have lipase domains or activity, it was important to first determine which lipases are critical to lipid catabolism. This research identified three lipase genes that may be responsible for limiting lipid accumulation in Yarrowia.

Y. lipolytica is an oleaginous yeast, with wild-type strains able to accumulate lipids to account for over 20% of its dry cell weight. Over 90% of these lipids are stored in the form of triacylglycerols (TAGs); biodiesel can be made from TAGs through transesterification with short chain alcohols to form fatty acid alkyl esters. Y. lipolytica is capable of consuming many different carbon sources, with acetate being one particularly attractive example as a major component of many agro-industrial or municipal waste streams.

Previously, our lab developed a strain of Yarrowia with increased expression of the native ACC1 and DGA1 genes in order to greatly improve flux towards lipid synthesis. In a bioreactor, this strain typically achieves a maximum lipid content (grams lipids per gram dry cell weight) between 55-65% when grown in minimal medium with acetate as a carbon source, with lipids being produced throughout the fermentation until an abrupt stationary phase is reached. MicroGC measurements for carbon dioxide in the bioreactor headspace during this stationary phase indicate that these cells are still metabolically active at that time, leading us to consider the possibility that the static lipid content during stationary phase could be the result of simultaneous lipid synthesis and degradation (which occur in separate cellular compartments) rather than a more physical limitation of the cell in being unable to store more lipids.

In the first step of lipid degradation in Yarrowia, a lipase or esterase removes fatty acids from TAGs or other glycerolipid molecules. Free fatty acids are then degraded through β-oxidation by the POX (1-5) enzymes into acetyl-CoA units, which can be oxidized in the TCA cycle to make ATP. We carried out a combination of literature review with the results of BLAST searches to find gene products in Yarrowia that are homologous to intracellular lipases from other yeasts. This search allowed us to identify 23 genes that could potentially be responsible for lipid mobilization. This number was further reduced by determining which of these genes may be the most responsible for lipid mobilization and therefore the best candidates for knockouts.

We took RNA samples of the ACC1- and DGA1-overexpressing strain growing in an acetate bioreactor at 2 days and 10 days to represent times during which the cells are in a lipid- producing growth phase and in stationary phase, respectively. We then performed what we will call a Differential Expression Analysis (DEA) on all 23 genes, comparing their expression levels at 10 days (relative to the constitutive actin gene) to their expression levels at 2 days (also relative to actin) to find genes that were most highly upregulated only when the cells have a high (~50-55%) lipid content. We have identified three genes, LIP1 (fold change: 12.1), LIP4 (fold change: 18.7), and LIP10 (fold change: 107.0), that showed large increases in expression during the lipid-static phase. In a ΔKu70 background engineered for us by collaborators at Pacific Northwest Nation Laboratory (PNNL), we have been attempting to create strains with a single lipase removed and a strain with all three of these lipases removed, and we will overexpress ACC1 and DGA1 in all of them to compare the contribution of each lipase to the process of lipid mobilization and achieve a lipid content of Yarrowia higher than what was originally possible.

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