Metabolic Engineering *Yarrowia lipolytica* to Produce 3-acetyl-1,2-diacyl-sn-glycerol

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Project Goals: The goal is to showcase a combination of metabolic engineering strategy to produce high-level 3-acetyl-1,2-diacyl-sn-glycerol. *Yarrowia lipolytica* QY06 strain devoid lipid droplet formation can serve as a platform strain to study lipid droplet bud-off mechanism and isolate high activity acTAG synthase.

The triacylglycerols 3-acetyl-1,2-diacyl-sn-glycerol (acTAG) have many potential industrial applications such as engine lubricant oil, emulsifiers, food coatings, and plasticizers. As an oleaginous yeast, *Yarrowia lipolytica's* high flux toward native TAG (lcTAG) synthesis serves as an excellent chassis for production of acTAG. The acTAG are unusual triacylglycerols (TAG) with an acetyl group at the sn-3 position instead of the typical long-chain acyl group. Compared to regular TAG, the acetyl group of acTAG confers useful physical and chemical properties such as reduced kinematic viscosity and lower melting points.

The main challenge here is how to rewire *Y. lipolytica's* native metabolism from lcTAGs to acTAGs. To accomplish this, the presented work provides a two-fold strategy: removing the competing pathways of lcTAG synthesis and identifying highly active acTAG synthases. In the first strategy, we generated a multi-knockout *Y. lipolytica* strain QY06 that deleted three acyltransferases, two lipases, and one dehydrogenase. This engineered strain is incapable of producing lcTAG and forming lipid droplets due to removal of three TAG synthase genes. In the second strategy, we evaluated the activities of 30 bioprospected acTAG synthase homologs in an engineered *Y. lipolytica*. An enzyme homolog from *Euonymus alatus* showed higher activity of acTAG synthesis. When evaluating the acTAG titer as function of lipid droplet content, it was observed that acTAG titer is proportional to the lipid droplet content (e.g. lcTAG titer), indicating acTAG localization in lipid droplet may help alleviating acTAG toxicity to cells by pulling it away from the cytosol and storing in the lipid droplet. Finally, we reported an engineered strain is capable of producing 12 g/L acTAG from glucose in a fed-batch fermentation experiment, and 4 g/L acTAG from sugarcane juice in shake flask experiments. We showcase that combination of metabolic engineering strategy such as blocking competing pathway, pulling flux towards acTAG synthesis, and alleviating toxicity by compartmentalization, to achieve high level production of acTAG.

References

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