METABOLIC ENGINEERING OF YEAST FOR INCREASED PRODUCTION OF CYCLOPROPANE FATTY ACIDS

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Biological production of chemicals and fuels using whole cells is an important and growing segment of manufacturing and among the various forms, microorganisms are the most successfully utilized. In particular, yeasts such as *Saccharomyces cerevisiae* are both widely used production organisms and metabolic models for oleaginous yeasts. Fatty acid-containing lipids are one example of moderate value, highly versatile chemicals produced by yeasts that are used in a broad range of industries for lubrication, cosmetics, fuels and polymers.

Production levels of standard fatty acids by yeasts has increased enormously over the past 10 years through the application of metabolic pathway engineering, flux analysis, computational approaches and to a lesser extent, bioprocessing improvements. Combined, these advances have brought yeast-based fatty acid production close to commercial reality. Functionalized fatty acids such as those containing hydroxyl or cyclopropyl groups are more valuable as chemical feedstocks and are an attractive target for yeast production as commercial supply is limited. Cyclopropane fatty acids, possessing a strained 3-membered ring and having a saturated chain, are especially attractive as they have application in cosmetics and specialty lubrication. However, cyclopropyl fatty acids present greater challenges for metabolic engineering as they are not produced naturally by yeast.

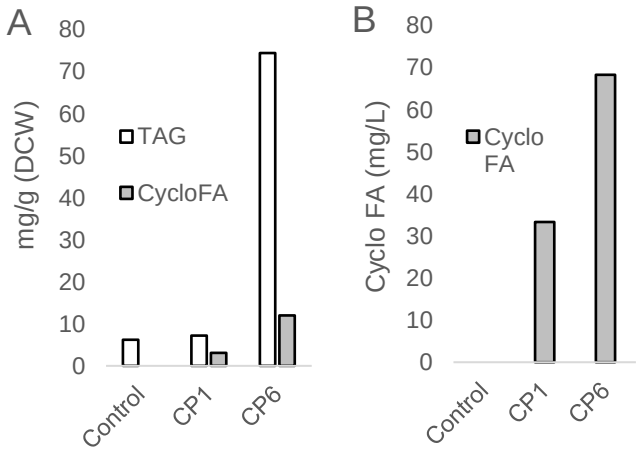

Figure 1. Production of triacylglycerol (TAG) or cyclopropyl fatty acids (cycloFA) in TAG on A) a dry cell weight basis (mg/g DCW) or B) in productivity terms (mg/L) in S. cerevisiae expressing EcCFAS only (CP1) or together with genes to increase standard fatty acids (CP6).

When we expressed the *Escherichia coli* cyclopropane fatty acid synthetase (EcCFAS) in *S. cerevisiae,* both *cis*-9,10-methylene-hexadecanoic and -octadecanoic acids were identified in the phospholipid and triacylglycerol fractions of the cell. Furthermore, EcCFAS expressed in cells engineered for increased lipid production through increases in fatty acid synthesis, accumulation and sequestration in lipid droplets, increased cyclopropyl fatty acid content 4-fold in triglyceride and yield increased to 68.3 mg/L (Fig 1 A& B; Peng et al. in press). This result is very promising for yeast production and there is great potential to improve content further as the triacylglycerol fraction had just 16% present as cyclopropyl fatty acid whereas phospholipid remained enriched at 40%. To further improve yield and purity of this fatty acid in yeast through metabolic engineering, we have undertaken a systematic study of location(s) of cyclopropane fatty acid synthesis, assessed the ability of native yeast enzymes to process the exotic fatty acid, examined potential substrate limitations and the heterologous expression of acyl handling genes. The outcomes of these approaches will be described and show the path towards improving the production of cyclopropyl and other similarly synthesized high value exotic fatty acids in yeasts.

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