

ENABLING MEDIUM-CHAIN FATTY ACID PRODUCTION IN YEAST VIA HIGH-THROUGHPUT MALDI MS-BASED ENZYME ENGINEERING

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Free fatty acids (FFAs) are considered important and valuable products, existing in crucial nutrients, soaps and fuels. Accordingly, the production of FFAs in fungi has been investigated extensively over years due to its metabolic capability. Particularly for controlling the fatty acid chain length compositions that can benefit formation of desired biofuels and chemicals. However, existing methods for production and selection of shorter-chain length FFAs are still time-consuming and labor-intensive. Here, we report a mass spectrometry (MS) based high-throughput screening method for detecting medium-chain fatty acids (MCFAs) in *Saccharomyces cerevisiae* by using lipids as a proxy. Four first shell key residues in the fatty acid synthase (FAS) are chosen to perform site-saturation mutagenesis and 288 colonies are screened to achieve 95% library coverage. The results from the Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) MS indicate improved shorter acyl-chain phosphatidylcholine (PC) production in ~100% of the mutants compare with the wild type. Finally, we identify several mutations among all four libraries of interest that can help with baker's yeast shorter acyl-chain length phospholipids production. Furthermore, we correlate the lipids with MCFAs productions by GC-MS characterization on selected candidates after sequencing check. The presented technology should be generally applicable for controlling fatty acid compositions in a high-throughput manner far beyond baker's yeast.

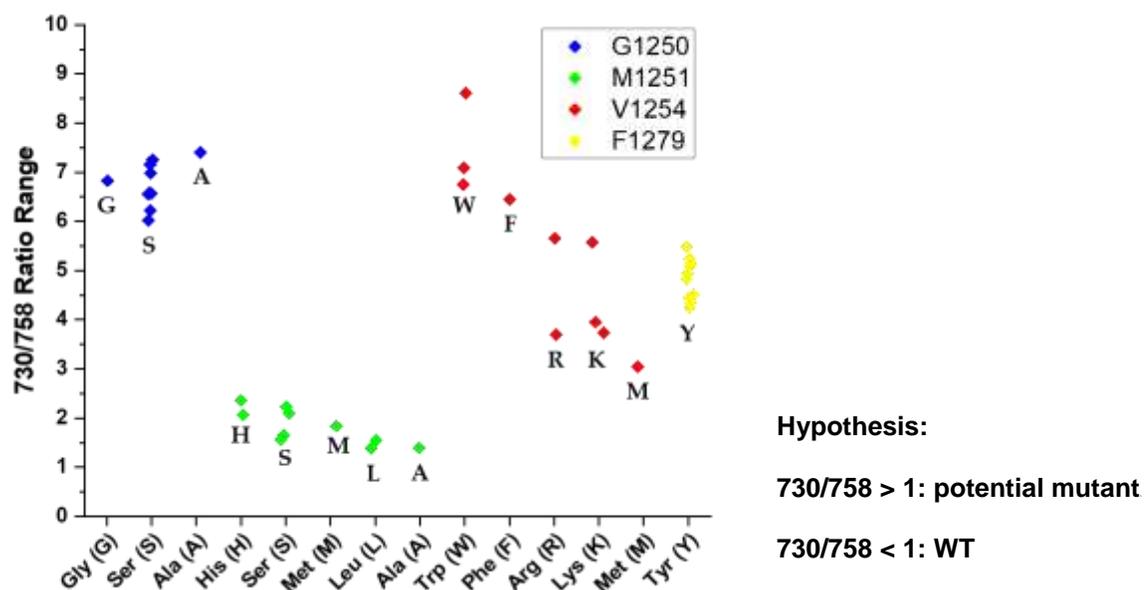


Figure 1 – Sequencing result of Top 10 730/758 m/z peak ratio colonies of all 4 libraries. Multiple different amino acids can help with increasing shorter acyl chain lipid production.