Commercial Production of Eicosapentaenoic Acid by Fermentation of Engineered Yeast

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Omega-3 fatty acids, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), mainly found in fish oil, have proven health benefits for both human and animals. Concerns over the quality and sustainability of the fish oil supply have generated interest in alternative sources for production of EPA and DHA. We report the development of a clean and sustainable source of omega-3 fatty acids by fermentation, using a metabolically engineered strain of the oleaginous yeast, *Y. lipolytica*. While certain strains of *Y. lipolytica* can accumulate oil up to 40% of the dry cell weight, the only PUFA normally synthesized by the organism is linoleic acid (C18:2n-6). Coordinate expression of desaturase genes and elongase genes comprising omega-3 fatty acid biosynthesis pathways were sufficient to demonstrate the synthesis of EPA. However, only an integrated strategy based on use of strong promoters, increase in gene copy numbers, push and pull of carbon into the engineered pathway, enhanced acyl-exchange and use of fermentation process conditions to increase oil production resulted in the generation of a high EPA production strain for commercialization. The yeast triacylglyceride oil has a unique fatty acid profile with greater than 55% as EPA, and less than 10% as saturated fatty acid. This metabolic engineering of *Yarrowia* generates a platform technology to produce tailored omega-3 or omega-6 fatty acid compositions. Our land-based production of EPA, DHA, ARA or GLA provides a superior source for these essential molecules for applications in nutritional supplements, functional foods, infant foods, pharmaceuticals, and animal feeds. We will also describe the importance of peroxisome in regulating fatty acid metabolism in engineered *Yarrowia* strains.