COMMERCIAL PRODUCTION OF OMEGA-3 FATTY ACIDS BY METABOLIC ENGINEERING OF *YARROWIA LIPOLYTICA*

Seung-Pyo Hong, Zhixiong Xue, Pamela Sharpe, Narendra Yadav, Xiaochun Fan, Bjorn Tyreus, David Short, Dongming Xie, Boonchai Boonyaratanakornkit, Clementina Dellomonaco, and Quinn Zhu

Central Research & Development, Biochemical Sciences & Engineering, BioMaterials, E.I. du Pont de Nemours and Company, Wilmington, DE 19880
Email: raymond.hong@usa.dupont.com

Key words: Omega-3, EPA, Yarrowia lipolytica

Introduction. Omega-3 long chain polyunsaturated fatty acids (LCPUFAs), especially eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), are natural products essential for human health (1). EPA and DHA also have a wide range of other uses including aquaculture, terrestrial animal feed, pet food, and personal care. The demand for them is growing rapidly. Most of the current EPA and DHA in commerce come from wild caught ocean fish. The increasing demand for EPA and DHA puts additional pressure on threatened ocean fisheries. We report a platform for sustainable, land-based commercial production of EPA from engineered yeast *Yarrowia lipolytica*.

Methods. The high EPA titer was achieved through carefully balanced expression of desaturases and elongases of the delta-9/ delta-8 pathway for polyunsaturated fatty acid biosynthesis and targeted modification of host fatty acid and lipid metabolism (2). Mutation in the *PEX10* gene led to a remarkable increase in EPA titer. Fluorescence and electron microscopy studies showed protein import into peroxisome was disrupted and peroxisome morphology and integrity were compromised in *pex10* strains.

Results. Metabolic engineering of oleaginous yeast *Yarrowia lipolytica* led to the generation of a strain capable of producing eicosapentaenoic acid (EPA) at 15% of its dry cell weight (DCW). This strain has been used commercially to produce EPA-rich oil and biomass. The yeast oil has a unique fatty acid profile with greater than 56% EPA and less than 5% saturated fatty acid, the highest and the lowest respectively among known EPA sources. The breakthrough discovery that peroxisome biogenesis gene *PEX10* disruption greatly increases EPA production and lipid accumulation is applicable for increased production of other lipid-related products. Our results demonstrate that genetic engineering of microorganisms is a commercially viable alternative to naturally isolated organisms for the production of high quality LCPUFAs in a sustainable fashion.

Conclusions. Until now, high levels of EPA could only be produced from fish oil by expensive separation and enrichment methods. Through genetic engineering, we have generated a *Y. lipolytica* strain Y4305 that made land-based commercial production of EPA possible for the first time. This breakthrough achievement was a result of careful selection of host organism and EPA production pathway, balanced expression of pathway enzymes, and modification of host metabolism to enhance lipid accumulation and remodeling. This developed *Yarrowia* technology platform provides a superior land-based sustainable source of EPA, and is capable of producing oils with tailored ω-3 or ω-6 fatty acid compositions.

Acknowledgements. The work was supported by E.I. du Pont de Nemours and Company. We also thank Ethel Jackson for her strong support.

References.

1. Deckelbaum RJ, Torrejon C. (2012), *J. Nutr.* **142**: 587S - 591S.

2. Zhu, Q., *et al.* (2010) Metabolic engineering of an oleaginous yeast for the production of omega-3 fatty acids. In: *Single cell oils: microbial and algal oils* (eds. Cohen, Z., Ratledge, C.), AOCS press, Urbana, 51–73.