

林果特异功能性脂肪酸的高效制备：培育还是培养？

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摘要：油脂是三大营养素之一，可在人体中发挥重要的生理功能，对于人体健康至关重要。为践行“大食物观”的理念，可以动物、草本种子、林果和产油微生物等自然资源为基础建立多元化的油脂供应体系。但不同来源的油脂因功能性脂肪酸的组成不同，具有不同的营养功能。作为一种超长链脂肪酸（Very long-chain fatty acid, VLCFA），神经酸（Nervonic acid, NA）在认知改善、大脑发育和脑部疾病治疗方面具有特定的生物学功能，因此受到广泛关注。早期传统 NA 的来源为鲨鱼中提取，但猎捕鲨鱼会破坏海洋生物系统，并在很多地区已经颁布相关政策法规禁止猎捕鲨鱼。且天然产 NA 的蒜头果、元宝枫和文冠果等木本植物也面临着生长条件苛刻和产油率低的缺陷。因此，采用食品合成生物学手段，在其它生物系统中实现 NA 的高产，是当前 NA 研究的热点。目前，植物和微生物代谢工程均实现了 NA 的异源生物合成，并有望通过培育转基因作物或培养工程菌株的方法实现 NA 的可持续生产。在植物代谢工程方面，3-酮酰辅酶 A 合酶（3-ketoacyl-CoA synthases, KCS）的种类、种子特异性启动子、转基因拷贝数、最佳基因组整合位点和足够的特异性底物，是提高 NA 含量的关键性因素。其中，转基因作物埃塞俄比亚芥（*Brassica carinata*）的 NA 占总脂肪酸的平均含量可达 42.2%，且每亩 NA 的产量约 27~32 Kg。与植物代谢工程相对比，微生物细胞工厂具有生长速度快、生产效率高、可控性强、无季节要求以及不占耕地的显著优势。产油酵母具有油脂含量高、鲁棒性强且易高密度发酵的特点，成为代谢改造微生物生产功能性脂肪酸和其衍生物的优良底盘。但代谢工程改造产油酵母高产 NA 依然存在如下问题：（1）C18:1 延伸为 C24:1 过程中，是否需要分别偏好不同底物的不同 KCS 的协同作用，从而提高脂肪酸延伸（Fatty acid elongation, FAE）模块的效率；（2）在油脂合成期，NA 与油脂积累之间的动态关系以及可能导致 NA 生物合成不足的酶基因尚未被探究；（3）缺乏有效的启动子元件在油脂合成期驱动关键基因的表达；（4）缺乏与高产 NA 工程酵母相匹配的发酵工艺。因此，以高产油脂的圆红冬孢酵母（*Rhodospiridium toruloides*）作为底盘，首先考察单独及组合表达分别偏好不同底物 KCSs 的编码基因对各 VLCFAs 合成的影响，从而初步构建能够合成 NA 的工程菌株；其次，通过发酵进程分析，揭示 NA 与油脂合成的动态关系，并通过转录组学的技术手段，比较细胞增殖期和油脂合成期转录组的变化，从而建立油脂积累、NA 合成和基因转录水平三者之间的关系，揭示 NA 合成途径的关键靶点基因；随后，根据细胞增殖期和油脂合成期的转录组数据，进一步挖掘油脂期激活的启动子，对 NA 和油脂合成的关键性步骤进行调节，并构建可合成 NA 的脱饱和支路；最后，通过发酵优化与过程控制，在发酵罐水平实现 NA 的高产。最终工程菌株经分批补料发酵后，NA 占总脂肪酸的百分比、滴度和时空产率分别达到 46.3%、44.2 g/L 和 0.230 g/L/h，均显著超过代谢工程改造植物和产油酵母当前报道的最高水平。

关键词：油脂；功能性脂肪酸；神经酸；代谢工程；超长链脂肪酸

Efficient preparation of specific functional fatty acids of forest fruits: breeding or cultivating?

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Abstract: Lipid is one of the three major nutrients that can perform important physiological functions in the human body, which is vital to human health. To adopt the concept of “all-encompassing approach to food”, a diversified lipid supply system can be built based on animal, herb seeds, forest fruit and oleaginous yeasts. Lipids from different sources in nature provide different nutritional features depending on the composition of their functional fatty acids. As a very long-chain fatty acid (VLCFA), NA has attracted considerable attention due to its specific biological functions in cognitive improvement, brain development, and therapies for brain diseases. NA was traditionally extracted from sharks in the early years, but shark hunting destroyed marine ecosystems and has been banned in many regions by relevant policies and regulations. Furthermore, the natural NA-producing woody plants such as *Malania oleifera*, *Acer truncatum* and *Xanthoceras sorbifolium* suffer from harsh growing conditions and low lipid-yield. Applying food synthetic biology to produce high-level of NA in other organisms is thus a research hotspot. Currently, the heterologous biosynthesis of NA was achieved by both plant and microbial metabolic engineering, which were expected to lead to the sustainable production of NA by breeding transgenic crops or cultivating engineered strains. The species of 3-ketoacyl-CoA synthases (KCS), seed-specific promoters, transgene copy numbers, optimal genome integration sites, and sufficient specific substrates are critical factors for increasing NA content in plant metabolic engineering. The average percentage of NA in total fatty acid from transgenic *Brassica carinata* was up to 42.2%, which could produce about 27~32 Kg of NA per acre. In contrast to metabolic engineering of plants, microbial cell factory possesses the significant advantages of rapid growth rate, high productivity, strong controllability, no seasonal requirements, and not occupying arable land. Oleaginous yeasts are excellent chassis to produce functional fatty acids and their derivatives by metabolic engineering, owing to its characteristics of high lipid content, strong robustness and high cell-density cultivation. However, the following problems still exist in the high NA yield of oleaginous yeast modified by metabolic engineering: (1) During the elongation of C18:1 to C24:1, whether the synergistic effect of multiple 3-ketoacyl-CoA synthases (KCSs) with different substrate specificity is required, so as to improve the efficiency of fatty acid elongation (FAE) module; (2) the dynamic relationship between NA and lipid accumulation, and the genes coding enzymes that may lead to insufficient NA biosynthesis during the lipogenic stage have not been explored; (3) lack of effective promoter elements to drive the expression of key genes during lipid synthesis; (4) lack of fermentation process matching with engineered yeast producing high-yield NA. Therefore, *Rhodospiridium toruloides* with high-yield lipid was used as the chassis in this work. Firstly, the effects of individual and combined expression of KCSs genes coding enzymes preferencing different substrate on the synthesis of VLCFAs were investigated, so as to preliminarily construct engineered strains capable of synthesizing NA. Secondly, the dynamic relationship between NA synthesis and lipid synthesis was revealed through the analysis of fermentation process, and the transcriptome changes between cell proliferation phase and lipogenic phase were compared through transcriptomic techniques, so as to establish the relationship between lipid accumulation, NA synthesis and gene transcription level, and reveal the key target genes of NA synthetic pathway. Then, according to the transcriptome data of cell proliferation phase and lipogenic phase, the activated promoter of lipogenic phase was further mined, and the key steps of NA and lipid synthesis were regulated, and the desaturation pathway for NA synthesis was constructed. Finally, the high yield of NA was

achieved at the fermenter level through fermentation optimization and process control. Through fed-batch fermentation by the final strain, the NA percentage of TFA, NA titer and NA productivity reached 46.3%, 44.2 g/L and 0.230 g/L/h, markedly exceeding the highest current reports of metabolically engineered plants and oleaginous yeasts.

Key words: Lipids; Functional fatty acids; Nervonic acid; Metabolic engineering; Very long-chain fatty acids