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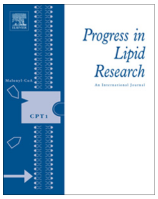

Review

Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids

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ABSTRACT

Omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs) have received growing attention due to their significant roles in human health. Currently the main source of these nutritionally and medically important fatty acids is marine fish, which has not met ever-increasing global demand. Microorganisms are an important alternative source also being explored. Although many microorganisms accumulate omega-3 LC-PUFAs naturally, metabolic engineering might still be necessary for significantly improving their yields. Here, we review recent research involving the engineering of microorganisms for production of omega-3 LC-PUFAs, including eicospentaenoic acid and docosohexaenoic acid. Both reconstitution of omega-3 LC-PUFA biosynthetic pathways and modification of existing pathways in microorganisms have demonstrated the potential to produce high levels of omega-3 LC-PUFAs. However, the yields of omega-3 LC-PUFAs in host systems have been substantially limited by potential metabolic bottlenecks, which might be caused partly by inefficient flux of fatty acid intermediates between the acyl-CoA and different lipid class pools. Although fatty acid flux in both native and heterologous microbial hosts might be controlled by several acyltransferases, evidence has suggested that genetic manipulation of one acyltransferase alone could significantly increase the accumulation of LC-PUFAs. The number of oleaginous microorganisms that can be genetically transformed is increasing, which will advance engineering efforts to maximize LC-PUFA yields in microbial strains.

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1. Introduction

Omega-3 very long-chain polyunsaturated fatty acids (ω3-VLCPUFAs), especially eicosapentaenoic acid (EPA, $20{:}5\Delta^{5,8,11,14,17}$) and docosahexaenoic acid (DHA, $22{:}6\Delta^{4,7,10,13,16,19}$) are considered to be essential for the proper visual and neurological development of infants [1–4]. They are also known to be positively associated with healthy aging throughout life, particularly by reducing the incidence of cardiovascular diseases in adults [5–7]. Considerable clinical and epidemiological evidence has indicated the therapeutic importance of ω3-VLCPUFAs in the prevention or treatment of many diseases including myocardial infarction, bronchial asthma, inflammatory bowel diseases [8,9], major depression [10,11] and several types of cancer [12]. The health benefits of ω3-VLCPUFAs for the human body have been reviewed recently [13]. DHA is an important component of membrane phospholipids of the brain, retina and spermatozoa [14]. EPA can be converted to eicosanoids, a group of biologically active chemicals including prostaglandin, thromboxane and leucotriene, which play crucial roles in regulation of blood pressure and blood coagulation and participate in many important physiological processes such as inflammatory and immunological reactions [15]. For humans, the most common dietary intake of essential PUFAs is linoleic acid (LA, $18{:}2\Delta^{9,12}$) and α-linolenic acid (ALA, $18{:}3\Delta^{9,12,15}$), both of which are primarily plant and animal-derived [16]. However, these PUFAs cannot provide the health benefits described above. Humans can convert linoleic acid (LA, $18{:}2\Delta^{9,12}$) and α-linolenic acid (ALA, $18{:}3\Delta^{9,12,15}$), the precursor of ω3-VLCPUFAs, to EPA and DHA. The conversion of ALA to ω3-VLCPUFAs in humans is based on the positive link between increased intakes of dietary ALA and enhancement of EPA and DHA in plasma and cell lipids [17,18] as well as the results of stable-isotope-tracer studies [19]. However, the estimated overall efficiency of conversion of ALA to EPA is about 5% and of ALA to DHA is <0.5% although the conversion is variable and tissue specific. The American Heart Association has recommended supplemental intake of ω3-VLCPUFAs (mostly as EPA and DHA) of about 1 g/day for patients with coronary heart disease (CHD), and 24 g/day for patients who need to lower the level of triglycerides. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) has recommended a minimum intake of EPA and DHA combined of 500 mg/day for cardiovascular health in adults. Supplementation of about 200 mg/day of omega-3 DHA is recommended for pregnant and lactating women by the World Association of Perinatal Medicine Dietary Guidelines Working Group, which is also a European recommendation supported by many health organizations and agencies. Unfortunately, median intakes of EPA and DHA in many countries are far below these recommended or suggested amounts [20]. For those people who have increased risk of cardiovascular disease and other related diseases or patients with these diseases, consumption of adequate amounts of ω3-VLCPUFAs (mostly as EPA and DHA) should be encouraged due to their multiple health benefits.

Currently marine fish and seafood are the most common dietary sources of ω3-VLCPUFAs, which are concentrated through the ocean food chains from ω3-VLCPUFA-synthesizing microorganisms (e.g. microalgae) [21]. However, fish oils are potentially unsustainable and unsafe and thus have not met the ever increasing global demand for ω3-VLCPUFAs because worldwide fish stocks are declining and environmental pollution of marine ecosystems has become a pervasive and global problem [22], which has led to increasing interest in the search for alternative sustainable sources of ω3-VLCPUFAs. There are two potential alternatives to fish oils: microbial single cell oils and vegetable oils from metabolically engineered plant oilseeds. Successful high-level accumulation of ω3-VLCPUFAs via metabolic engineering of plant oilseeds has been reported in several species including *Brassica juncea* [23], *Arabidopsis thaliana* [24] and *Camelina sativa* [25]. Wu et al. reported that an EPA level of up to 15% of total seed fatty acids was achieved in *B. juncea* by using a series of transformations with increasing numbers of transgenes [23]. Ruiz-Lopez et al. used an iterative approach to optimize the accumulation of ω3-VLCPUFAs in transgenic *Arabidopsis* seeds and their efforts demonstrated high levels of EPA (up to 13% of total seed fatty acids) and DHA (with the average yield of 2.5% of total seed fatty acids) [24]. Subsequently, the same authors also described the successful reconstruction of the EPA and DHA biosynthetic pathway in the seeds of an oilseed crop, *C. sativa*. They successfully engineered this species to accumulate high levels of ω3-VLCPUFAs in its seed oils and yielded two iterations, in which one accumulated EPA up to 31% of the seed oils and the other accumulated up to 12% EPA and 14% DHA in the seed oils [25]. These research efforts demonstrate the feasibility of large-scale production of ω3-VLCPUFAs in the oilseeds of transgenic crops, which have proved to be a promising alternative to fish oils.

Although transgenic plants have several advantages for production of ω3-VLCPUFAs such as high efficiency of oil accumulation, well-developed molecular tools for genetic manipulation, low cost of obtaining target fatty acids from oilseeds and absence of either an unpleasant odor or high amounts of cholesterol, their production is highly associated with season, climate change, available arable land, and public concerns on transgenic crops that are cultivated in open ecosystems. In particular, production of transgenic oilseed crops at large scale in agriculture has not been well-accepted in many countries throughout the world, especially in Europe and China [26]. Regulatory issues and societal opposition regarding commercial use of genetically modified (GM) crops might lead to delay in the commercial application of GM-crop-derived ω3-VLCPUFAs. Microorganisms are known to be natural producers and the original sources of ω3-VLCPUFAs. Despite the high production cost, microorganisms offer a few advantages for the production of ω3-VLCPUFAs via metabolic engineering strategy including higher growth rates, requirement of simple nutrient input, controllable culture condition, simple fatty acid composition, easy genetic manipulation and well-annotated genomes and metabolic pathways. Microbial oils usually contain a significant amount of natural antioxidants such as carotenoids and tocopherols, which play a role in protecting ω3-VLCPUFAs from oxidation. Additionally, many microbial strains can also produce other high-value compounds such as squalene and phytosterols that offer additional benefits to human health.

The overall production cost may be largely reduced by biorefinery. Microbial fermentation is generally conducted in a closed production system and it can be scaled up to a commercial level. Nowadays a few microbial species that naturally produce EPA and/or DHA have been commercially explored and utilized. For example, the heterotrophic microalga *Crypthecodinium cohnii* and the protist *Scizochytrium* sp. have been approved to commercially produce DHA mainly used for pharmaceutical products and infant formula, which represent an important industrially alternative source of DHA [27,28]. Although a few microbial species/strains are natural producers of ω3-VLCPUFAs, the yields of target fatty acids derived from these microorganisms are relatively low and the costs for their cultivation are high. However, with the advent

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