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Review

Advancing oleaginous microorganisms to produce lipid via metabolic engineering technology

Ming-Hua Liang [a], Jian-Guo Jiang [a,b,*]

[a] School of Biological Science & Engineering, South China University of Technology, Guangzhou 510006, China
[b] College of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China

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A B S T R A C T

With the depletion of global petroleum and its increasing price, biodiesel has been becoming one of the most promising biofuels for global fuels market. Researchers exploit oleaginous microorganisms for biodiesel production due to their short life cycle, less labor required, less affection by venue, and easier to scale up. Many oleaginous microorganisms can accumulate lipids, especially triacylglycerols (TAGs), which are the main materials for biodiesel production. This review is covering the related researches on different oleaginous microorganisms, such as yeast, mold, bacteria and microalgae, which might become the potential oil feedstocks for biodiesel production in the future, showing that biodiesel from oleaginous microorganisms has a great prospect in the development of biomass energy. Microbial oils biosynthesis process includes fatty acid synthesis approach and TAG synthesis approach. In addition, the strategies to increase lipids accumulation via metabolic engineering technology, involving the enhancement of fatty acid synthesis approach, the enhancement of TAG synthesis approach, the regulation of related TAG biosynthesis bypass approaches, the blocking of competing pathways and the multigene approach, are discussed in detail. It is suggested that DGAT and ME are the most promising targets for gene transformation, and reducing PEPC activity is observed to be beneficial for lipid production.

Contents

Abbreviations: ACC, acetyl-CoA carboxylase; ACL, ATP:citrate lyase; ACP, acyl-carrier protein; AGPase, ADP-glucose pyrophosphate; AOX, acyl-CoA oxidase; ARA, arachidonic acid; BE, branching enzymes; CDP-DAG, CDP-diacylglycerol; DAG, diacylglycerol; DGAT, diacylglycerol acyl-transferase; DHA, docosahexenoic acid; DHAP, dihydroxyacetone phosphate; DHAPAT, DHAP acyltransferase; EPA, eicosapentaenoic acid; FAS, fatty acid synthetase; FAT, acyl-ACP-thioesterase; FFA, free fatty acid; GAP, glyceraldehyde 3-phosphate; GLA, gamma-linolenic acid; G-1-P, glucose 1-phosphate; G-6-P, glucose 6-phosphate; G3P, glycerol-3-phosphate; GPAT, glycerol-3-phosphate acyltransferase; *GPD1* and *GUT2*, glycerol 3-phosphate dehydrogenase; KAS, β-ketoacyl-ACP synthase; LPA, lysophosphatidate; LPAT, lysophosphatidate acyl-transferase; MAT, malonyl-CoA:ACP transacetylase; ME, malic enzyme; PA, phosphatidate; PAP, phosphatidic acid phosphatase; PDAT, phospholipid:diacylglycerol acyltransferase; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; Pi, inorganic pyrophosphate; PYC, pyruvate carboxylase; SS, starch synthase; TAG, triacylglycerol; WS/DGAT, wax ester synthase/acyl-CoA:diacylglycerol acyltransferase.

* Corresponding author at: College of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China. Tel./fax: +86 20 87113849.
E-mail address: jgjiang@scut.edu.cn (J.-G. Jiang).

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1. Introduction

The earliest research on producing lipids from microorganisms could be traced back to the First World War, when Germany had prepared with some strains of *Endomyces* and *Fusarium* sp. to produce lipids to solve the cooking oil shortage problem [1]. In recent years, high energy prices, energy and environment security, concerns about petroleum supplies are drawn great attention and drive us to find a renewable biofuel. One of the most promising renewable biofuels is biodiesel, a mixture of fatty acid methyl esters, and generally speaking, it is produced from vegetable oils, animal fats or wasting oils [2] by transesterification of triacylglycerols (TAGs) with short chain alcohols.

The global markets for biodiesel are entering a period of rapid and transitional growth. In the year 2007, there were only 20 nations producing biodiesel for the needs of over 200 nations; by the year 2010, more than 200 nations became biodiesel producing nations and suppliers. Global biodiesel production has massively increased to 18.2 billion liters per year from 2000 to 2010 (Fig. 1). However, the plant oil materials require large energy and acreage for sufficient production of oilseed crops. For example, using the average oil yield per hectare from various crops, the cropping area needed to meet 50% of the US transport fuel needs is shown in Fig. 2. This area is expressed as a percentage of the total cropping area of the US. In view of Fig. 2, microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel. If plant oil was used for biodiesel production, the cost of source would account for 70–85% of the whole production cost [3]. On the other hand, animal fat oils need to feed these animals. So the cost of oil feedstocks limits the large-scale development of biodiesel to a large extent. Therefore, to lower the cost of oil raw materials, much attention has been paid to the development of microbial oils and it has been found that many microorganisms, such as microalgae, yeast, bacteria, and fungi, have the ability to accumulate oils under some special cultivation conditions.

Compared to plant oils, microbial oils have many advantages, such as short life cycle, abundant and cheap raw materials, less labor required, less affections by venue, season and climate, and easier to scale up [8,9]. Therefore, microorganisms might become one of the potential oil feedstocks for biodiesel production in the future, although there are many researches associated with microorganism-producing oils that need to be further carried out. This review is covering the related researches about different oleaginous microorganisms for lipids production and microbial TAG biosynthesis process. Moreover, metabolic engineering strategies to increase lipid production are introduced in detail.

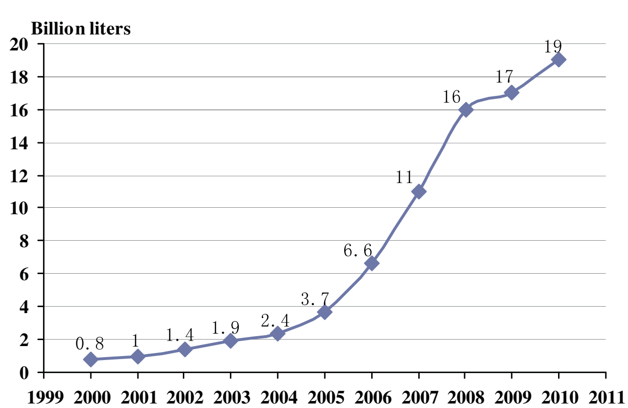

Fig. 1. Global biodiesel production in 2000–2010 [4].

2. Oleaginous microorganisms for biodiesel production

Oleaginous microorganisms are defined as oleaginous species with oil contents excess of 20% biomass weight [10]. Microbial oils, also called single cell oils, are produced by some oleaginous microorganisms, such as yeast, fungi, bacteria, and microalgae [8]. While the eukaryotic yeast, mold and microalgae can synthesize TAGs, which are similar with the composition of vegetable oils, and prokaryotic bacteria can synthesize specific lipids. Many oleaginous microorganisms can accumulate oils, especially TAGs, which are the main materials for biodiesel production. TAGs act with alcohol under acid or alkali catalyst by transesterification to generate fatty acid methyl ester (biodiesel) and the by-product glycerol [11] (Fig. 3).

Generally speaking, microbial oils might become one of the potential feedstocks for biodiesel production in the future. To reduce the cost of microbial oils, exploring other carbon sources instead of glucose is very important especially for such oils applied to biodiesel production. It was reported that xylose, glycerol, corn straw, and other agricultural and industrial wastes could be used as the carbon sources for microbial oils accumulation. Due to the low