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Recent advances and challenges of the use of cyanobacteria towards the production of biofuels

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ABSTRACT

Higher oil prices and the necessity for long-term energy security have increased the public and scientific attention on the production of biofuels. Bioenergy is much cleaner, safer, and more economical source of energy than fossil-based fuels. Of several organisms, cyanobacteria are attractive source of biofuels because of their genetic tractability, photosynthetic capability and lack of dependency on fertile land. Synthetic biology and metabolic engineering approaches have been successfully used towards the production of biofuels including ethanol, butanol, biodiesel and hydrogen. This review highlights the recent advances of pathway engineering and uses of synthetic biology tools in cyanobacteria for the production of economical and ecologically biofuels.

Contents

Abbreviations: ACC, Acetyl-CoA carboxylase; Adh, Alcohol dehydrogenase; Bldh, Butyraldehyde dehydrogenase; CRISPR, clustered regularly interspaced short palindromic repeats; DAG, Diacylglycerol; FFA, Free fatty acids; HydA, Hydrogenase; IspS, Isoprene synthase; Pdc, Pyruvate decarboxylase; PHB, Poly-β-hydroxybutyrate; RBS, Ribosome binding site; RuBisCO, Ribulosebisphosphate carboxylase/oxygenase; SD, Shine-Dalgarno; TAG, Triacylglycerol; TF, Transcription factor

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1. Introduction

The global consumption of fossil-based fuels has continued increasing, while the planetary reservoir is significantly depleting. Increased fuel consumption causes more environmental pollution, risks for human health and global warming. Thus, a need arises to address the current energy and environmental issues to produce biofuels. In order to reduce the price of oil, biofuels have become an alternative source over the traditional energy sources. Biofuels are also cleaner, safer and more eco-friendly, and importantly can be used directly in exiting internal combustion engine without modification [1]. In recent years, biofuels have been produced and used in Europe, the U.S., Brazil and in some Asian countries. As one of the major biofuels, ethanol has been produced through the fermentation process and the production had reached 9.2 billion gallons in the US in 2008, and has further increased by over 40% since 2007 [2]. In 2010 [3] ethanol production had increased to 13.2 billion gallons, contributing firmly to the major biofuel sources in current use. Now, the global ethanol production has risen up to 22.6 billion US gallons in which 90% of production is contributed by the U.S. and Brazil.

Initially, a major focus on ethanol production has been based on sugarcane (in Brazil) or grain, including corn in the US and wheat or wheat corn mixes in Canada and some parts of the European Union. In contrast, biodiesel is produced from oil seed crops (rapeseed, canola, soybean, sunflower in North America and the EU; soybean and favabean in Brazil) or palm oil (in many parts of south Asia). However, there remains no commercially available cellulosic biofuel production plant in North America or Europe. Whereas, a cellulosic ethanol plant was supposed to come on line in Italy in the fall of 2013, but so far this has not reached delivery. The common lignocellulosic feedstocks such as rapeseed, sunflower, switch grass, wheat, peanuts, sesame seeds and soybean are used to generate several liquid forms of energy, including alcohols (ethanol, propanol and butanol) and vegetable oils [4,5].

Irrespective of their successes, the major constraints of these energy crops are concerns of food security, land shortage and their need for irrigation. The production of biofuels from food and waste products also requires the processing of renewable substrates at the competitive prices. It may be possible to overcome this limitation by performing biofuel production from a non-food biomass source for energy supply by the use of photosynthetic organisms including cyanobacteria and algae. Even though biofuels are currently more expensive than the fossil-based fuels, their worldwide production is increasing exponentially [5] with the intention to become cost-effective in the near future. Cyanobacteria are photosynthetic prokaryotic microorganisms that use the sunlight, H_2O and CO_2 to produce their energy and storage components that form a potential feedstock that could generate bioenergy [5].

Recently, cyanobacteria are an attractive and alternative source for the production of biofuels because of their photosynthetic ability, tractable genetics, fast growth and independency of fertile land [6]. Cyanobacteria are significantly involved in the global carbon cycle, accounting for 20–30% of the Earth's photosynthetic productivity that converts solar energy into biomass [7]. *Synechocystis* sp. PCC6803 is the first cyanobacterium whose genome has been completely sequenced [8] and is considered to be an important model organism [9], allows us better understanding of its genetics and molecular mechanisms. However, cyanobacteria do not contain the complete biosynthetic pathway for the production of ethanol and butanol. Therefore, in order to produce these biofuels, we would need to use synthetic biology and metabolic engineering approaches for industrial scale production of biofuels at competitive prices.

In the past decade, synthetic biology has gained more scientific attention and interest towards the engineering of biological systems. Significant progress has been made on synthetic parts, device and circuits such as promoters [10–12], regulatory proteins and small regulatory RNAs [13–16], and scaffolds [17,18]. These parts are assembled in a wide range of hosts for construction of oscillators [19–21], riboregulators [15], riboswitches [22–24], biologic gates [25,26] and toggle switches [27]. Yet few genetic parts, devices and circuits have been designed and characterized in cyanobacteria; with the aim to tune, enhance and sustain the biofuels production. In a recent year, a number of research groups have been focused on cyanobacterial biotechnology for solar production and the enhancement of biofuel using metabolic engineering and synthetic biology approaches [28–30]. Recently, free fatty acids (FFAs) have also gained more scientific attention as potential precursors and targets for biofuels [31,32]. For example, diacylglycerol (DAG) and triacylglycerol (TAG) are storage compounds that could be used towards biofuels [33–35]. Additionally, a number of cyanobacterial strains were engineered for enhancing the production of hydrogen by through insertion of the hydrogenase gene [5,36,37]. Therefore, synthetic biology offers and permits the engineering of cyanobacteria for tuneable and controllable production of biofuels in a cost-effective manner. The aim of this review is to describe the recent advances and efforts using synthetic biology and metabolic engineering to engineer and characterize cyanobacteria towards the production of biofuels at industrial scales.

2. Growth conditions, cultivation and maintenance of cyanobacteria

Cyanobacteria generally grow *in vitro* in BG11 media at 30 °C, with continuous shaking and illumination of 50 mmol photons m^{-2}/s [38]. It captures the energy from sunlight, utilize atmospheric CO_2 and water during the photosynthesis process that converts inorganic substances into simple sugars [1,39]. These key factors verify the growth rate of cyanobacteria – light, temperature, culture medium, aeration, pH, CO_2 requirements, light and dark periods – with some of the nutrients (NaCl, $NaNO_3$, $MgSO_4$, $CaCl_2$, KH_2PO_4) citric acid and trace elements [1]. Naturally growing cyanobacteria however are more useful for large-scale production of biofuels rather than *in vitro* culture. Thus, culture and maintenance of cyanobacteria are preferable in large, shallow ponds, tanks, circular ponds and raceway ponds [40–42].

A major advantage exists for the cultivation of cyanobacteria in open ponds, which are easier to build and operate than the closed systems [43], although the former has some limitations, such as