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Review

Cyanobacterial chemical production

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ABSTRACT

The increase in global temperatures caused by rising CO_2 levels necessitates the development of alternative sources of fuel and chemicals. One appealing alternative that has been receiving increased attention in recent years is the photosynthetic conversion of atmospheric CO_2 to biofuels and chemical products using genetically engineered cyanobacteria. This can help to not only provide an alternate "greener" source for some of the most popular petroleum based products but it can also help to reduce atmospheric CO_2. Utilizing cyanobacteria rather than plants allows for reduced land requirements and reduces competition with food crops. This review discusses advancements in the field since 2012 with a particular emphasis on production of hydrocarbons.

Contents

1. Introduction

Many products used in daily life, from gasoline for cars to nylon and plastics, are derived from fossil fuels. In order to meet current energy and climate change challenges we must develop alternatives to fossil fuels as sources of energy and chemical production. When searching for an alternative to fossil fuels there are several important factors to consider: the efficiency of chemical production, cost, environmental impacts, and resources required (Keasling, 2012; Nielsen et al., 2014).

One promising avenue currently being explored is microbial chemical production. A great deal of work has been done to establish biofuel production in well known fermentative organisms such as yeast and *Escherichia coli* (Gronenberg et al., 2013; Jensen and Keasling, 2014; Rabinovitch-Deere et al., 2013). While this method of chemical production is a significant improvement over traditional fossil fuels, production in these host strains requires the addition of a carbon source, often sugar. This carbon source comes from plants, and therefore takes land and resources away from food crops, driving up the price of production (Tirado et al., 2010). Waste

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Table 1
Chemical production in cyanobacteria.

Product	Titer/Productivity	Host	Additional carbon source	Reference
2-methyl-1-butanol	0.2 g/L	7942		(Shen and Liao, 2012)
Isopropanol	0.1 g/L	7942		(Hirokawa et al., 2015)
23BDO	2.4 g/L	7942		(Oliver et al., 2013)
23BDO	3 g/L	7942	glucose	(McEwen et al., 2016)
Fatty alcohols	3 mg/gDCW	6803		(Yao et al., 2014)
Ethanol	2.3 g/L	6803		(Luan et al., 2015)
3HB	0.5 g/L	6803		(Wang et al., 2013a)
3HP	0.7 g/L	7942		(Lan et al., 2015)
P3HB	5% DCW	7002		(Zhang et al., 2015)
P3H4HB	4.5% DCW	7002		(Zhang et al., 2015)
Ethylene	5.7 mL/L/h	6803		(Ungerer et al., 2012)
Ethylene	0.9 mL/L/h	6803	xylose	(Lee et al., 2015)
Heptadecane	4 μg/gDCW	15041c		(Yoshino et al., 2015)
Free fatty acids	0.2 g/L	6803		(Liu et al., 2011)
Sucrose	0.1 g/L	7942		(Ducat et al., 2012)
Sucrose	0.1 g/L	6803		(Du et al., 2013)
GG	0.3 g/L	6803		(Tan et al., 2015)
Squalene	0.7 mg/OD/L	6803		(Englund et al., 2014)
13R-manoyl oxide	0.5 mg/gDCW	6803		(Englund et al., 2015)
Farnesene	70 μg/L/OD/day	7120		(Halfmann et al., 2014)
Isoprene	0.3 mg/gDCW	6803		(Bentley et al., 2014)
Lactic acid	0.8 g/L	6803		(Angermayr et al., 2014)
Lactic acid	2.2 g/L	6803	acetate	(Varman et al., 2013b)
Isobutanol	0.3 g/L	6803	glucose	(Varman et al., 2013a)

from plant crops in the form of lignocellulosics has been explored as a carbon source in these systems (Tilman et al., 2009). While advantageous in eliminating the food versus fuel land use dilemma, it is often difficult to break down these waste products into a usable form. Enzymes or high temperature and pressure conditions are often required, which adds to the cost of production (Sanderson, 2011).

Photosynthetic microorganisms offer an appealing alternative to traditional production hosts (Berla et al., 2013; Nozzi et al., 2013). With the use of a photosynthetic host it is possible to eliminate the need for added carbon and instead fix atmospheric CO_2 (Desai and Atsumi, 2013; Gronenberg et al., 2013). This also provides the added benefit of combating some of the causes of global climate change by the reduction of greenhouse gases. Without the need for added carbon, photosynthetic hosts such as algae and cyanobacteria do not require arable lands and could be grown in desert areas where they would not compete for land with food crops.

Several different cyanobacterial host species have been investigated for use in chemical production (Ducat et al., 2011; Oliver and Atsumi, 2014). A number of cyanobacterial strains have been shown to be amenable to genetic manipulation and the metabolic engineering tools available for such strains are increasing as interest in engineering cyanobacteria grows. Chemical production has already been demonstrated in cyanobacteria. In our previous review (Machado and Atsumi, 2012), we discussed some examples for cyanobacterial chemical production including isobutyraldehyde (Atsumi et al., 2009), isobutanol (Atsumi et al., 2009), 1-butanol (Lan and Liao, 2011), isoprene (Lindberg et al., 2010) and fatty acids (Liu et al., 2011). This shows the promise of cyanobacterial chemical production for wide scale use. However, there are several challenges to overcome before the goal of large-scale chemical production from cyanobacteria can be realized (Nozzi et al., 2013). These challenges include establishing tight control of gene expression, improving titers and removing bottlenecks, and expanding beyond established pathways (Boyle and Silver, 2012; Camsund and Lindblad, 2014; Marcheschi et al., 2013). This review discusses the progress made since 2012 (Machado and Atsumi, 2012) towards overcoming these obstacles with a focus on hydrocarbon production (Table 1).

2. New production pathways in cyanobacteria

One bottleneck in the broad use of cyanobacterial chemical production is the limited number of chemicals that can be produced. In recent years a great deal of work has been done to broaden the diversity of these chemicals and expand on the utility of cyanobacteria (Table 1).

2.1. Alcohols and diols

A wide variety of pathways for the production of industrially important chemicals have been developed in recent years. A prominent area of chemical production in cyanobacteria is the production of alcohols and diols. Alcohols and diols are both popular for use as biofuel candidates. Alcohols that have already been produced in engineered cyanobacteria include 1-butanol and isobutanol (Atsumi et al., 2009; Lan and Liao, 2011).

As mentioned in our previous review (Machado and Atsumi, 2012), production of isobutanol is reliant on the valine biosynthesis pathway (Atsumi et al., 2009). The intermediate 2-ketoisovalerate is funneled away from valine production and redirected to isobutanol through the activities of the heterologous enzymes 2-ketoacid decarboxylase and alcohol dehydrogenase (Fig. 1). Similar approaches have been applied to produce 2-methyl-1-butanol in cyanobacteria (Shen and Liao, 2012). 2-Methyl-1-butanol is derived from isoleucine biosynthesis and instead utilizes the intermediate 2-keto-3-methylvalerate (Fig. 1). A 2-ketoacid decarboxylase and alcohol dehydrogenase along with the citramalate pathway were utilized to produce 2-methyl-1-butanol in *Synechococcus elongatus* PCC 7942 (7942) with a final titer of 200 mg/L (Shen and Liao, 2012).

Isopropanol is of great interest because it can be readily converted into propylene and then to polypropylene, an important industrial material (Kusakabe et al., 2013). The addition of the isopropanol pathway (Fig. 2) from *Clostridium* (an acetyl transferase, acetyl-CoA acetyl transferase, acetoacetate decarboxylase, and a secondary alcohol dehydrogenase) in 7942 produced 27 mg/L after 9 days (Kusakabe et al., 2013). Building on this work by optimization of light conditions and buffering of the growth media helped to achieve an increase in production from 27 mg/L to 146 mg/L (Hirokawa et al., 2015).