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Photosynthetic approaches to chemical biotechnology

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National interest and environmental advocates encourage alternatives to petroleum-based products. Besides biofuels, many other valuable chemicals used in every-day life are petroleum derivatives or require petroleum for their production. A plausible alternative to production using petroleum for chemical production is to harvest the abundant carbon dioxide resources in the environment to produce valuable hydrocarbons. Currently, efforts are being made to utilize a natural biological system, photosynthetic microorganisms, to perform this task. Photosynthetic microorganisms are attractive to use for biochemical production because they utilize economical resources for survival: sunlight and carbon dioxide. This review examines the various compounds produced by photosynthetic microorganisms.

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Introduction

National interest and environmental advocates encourage alternative methods to petroleum-based products. Advances in metabolic engineering provide powerful tools to utilize microorganisms in the production of several useful chemicals. Photosynthetic organisms are beginning to be focused upon because they require very few substances to survive on: mainly light, carbon dioxide and water, making them beneficial and economical candidates for high volume production. Additionally, photosynthetic organisms do not occupy arable land space as they do not require crop feedstock for survival [1]. Aside from sustainability and renewable resource prospects, microbes are utilized to produce health related compounds [2,3].

The most common photosynthetic microorganisms used for the production of sustainable and high value compounds are cyanobacteria and algae. In many studies, heterologous genes are expressed in these microorganisms to improve productivity or to produce the compound of interest. First, heterologous genes are identified, which are then amplified via PCR or synthesized for codon optimization. Next, the pathway is constructed by arranging the genes under a number of citrons or operons where gene expression is constitutively active or induced. After introduction of this pathway into the host strain, the target chemical production is optimized by applying metabolic engineering methods such as removing competing pathways or improving enzyme activity.

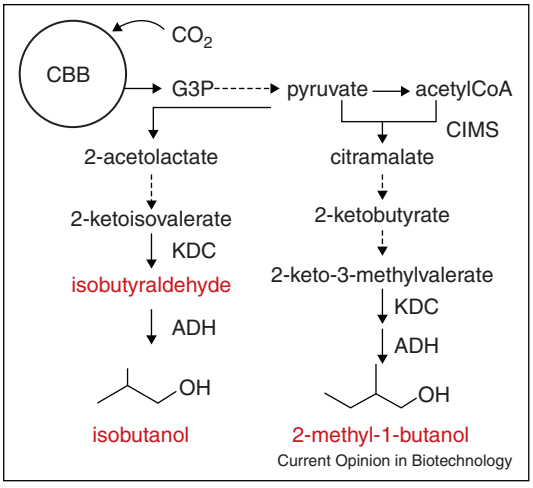
Recent advances in the ability to genetically manipulate [4,5•,6•] and characterize metabolic pathways in photosynthetic bacteria such as cyanobacteria [7] and purple photosynthetic bacteria [8] open the gateway for metabolic engineering. Through attempts to optimize metabolic pathways researchers have attempted to increase carbon fixation [9] which translates to an increased amount of carbon building blocks in the cell and thus the possibility for greater productivity. It has been found that greater carbon fixation occurs by increasing the expression of carboxysomes [10] or heterologous expression of Rubisco genes (*rbcLS*) [11]. In microalgae, attempts to increase carbon dioxide fixation have been made by creating a hybrid Rubisco enzyme which contains both plant and microalgae subunits [12].

Biofuels and bulk chemicals

Photosynthetic organisms are attractive candidates for biofuel production because they require inexpensive resources for survival; however, they do not naturally produce biofuels in high titers. Biofuel production in photosynthetic organisms has become more feasible with the help of metabolic engineering and synthetic biology. Ethanol is a well-known biofuel and was produced in titers of 240 mg/OD_{730}/L/day by introducing heterologous pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) in the photosynthetic bacterium, *Synechocystis* sp. PCC 6803 [13].

Isobutyraldehyde is a volatile compound that can be easily recovered from the media by gas stripping which decreases toxic compounds in the growth media and therefore does not stunt bacterial growth. In *Synechococcus elongatus* PCC 7942 the valine biosynthesis pathway was optimized to produce isobutyraldehyde (Figure 1). This was achieved by introducing the ketoacid decarboxylase gene (*kivd*) from *Lactococcus lactis* into the genome of *S. elongatus* PCC 7942 so that it could convert 2-ketoisovalerate to isobutyraldehyde. To improve the flux to 2-ketoisovalerate the *alsS* gene from *Bacillus subtilis* and the *ilvC* and *ilvD* genes from *Escherichia coli* were also inserted

Figure 1



2-Keto acid based biosynthesis pathways for isobutyraldehyde, isobutanol, and 2-methyl-1-butanol. Dot arrowheads contain multiple steps. CBB, Calvin–Benson–Bassham cycle; G3P, glyceraldehyde-3-phosphate; KDC, 2-ketoacid decarboxylase; ADH, alcohol dehydrogenase; CIMS, citramalate synthase.

into the chromosome. To improve the overall productivity of isobutyraldehyde, carbon fixation was improved in *S. elongatus* by the overexpression of Rubisco genes. This led to an isobutyraldehyde production rate of 6,230 μg/L/h. Installing YqhD, an E. *coli* alcohol dehydrogenase allowed for the production of isobutanol in titers of 450 mg/L in six days (Figure 1) [11]. In *S. elongatus* PCC 7942, 2-methyl-1-butanol (2MB) was produced at a rate of 2 mg/L/day by overexpressing heterologous enzymes for the citramalate pathway [14] to direct the pyruvate flux into the isoleucine synthesis pathway, where 2-keto-3methylvalerate was converted into 2MB by Kivd and YqhD (Figure 1) [15•].

1-Butanol is also a gasoline substitute and chemical feedstock, which is naturally produced in several *Clostridium* species and was produced anaerobically in the dark in engineered *S. elongatus* PCC 7942 strain at a titer of 14.7 mg/L in seven days (Figure 2) [16]. This was achieved by heterologous expression of four genes encoding acetyl transferase (AtoB) from *E. coli*, 3-hydroxybutyryl-CoA dehydrogenase (Hbd) from *Clostridium acetobutylicum*, trans-2-enoyl-CoA reductase (Ter) from *Treponema denticola*, crotonase (Crt) from *C. acetobutylicum*, and a bifunctional aldehyde/alcohol dehydrogenase (AdhE2) from *C. acetobutylicum*. 1-Butanol was produced in *S. elongatus* PCC 7942 aerobically by overcoming a thermodynamically unfavorable reaction, the condensation of acetyl-CoA, by ATP consumption and carbon dioxide evolution (Figure 2) [17•]. An ATP dependent malonyl-CoA biosynthesis enzyme, NphT7 was installed to utilize acetyl-CoA and begin substrate flux towards 1-butanol. To further optimize product yield, NADH dependent enzymes were replaced with NADPH dependent enzymes, because NADPH is a more abundant co-factor in cyanobacteria, to achieve 29.9 mg/L 1-butanol in a 20-day production. 2,3-Butanediol (23BD) is used in an array of products such as inks and fumigants and it can also be converted into a liquid fuel additive or industrial solvent. In *S. elongatus* PCC 7942, 2.38 g/L of 23BD was produced in a 21-day production. This was achieved by heterologous enzymes acetolactate synthase, 2-acetolactate decarboxylase (ALDC) and secondary alcohol dehydrogenase (sADH) [18•].

Isoprene is a volatile, five-carbon compound that is utilized in the synthesis of a variety of compounds from rubber to perfumes. Isoprene quickly evaporates from the media, which avoids toxicity issues for the microbe and is sequestered in the gas phase. Isoprene synthase gene, *ispS* from the vine *Pueraria montana*, was introduced into *Synechocystis* sp. PCC 6803 to utilize the naturally occurring methyl-erythritol-4-phosphate (MEP) pathway to produce up to 50 μg isoprene/dry cell weight (DCW)/day (Figure 3) [19]. Ethylene is another volatile compound that is a chemical feedstock used globally; its production from petrochemicals emits a lot of carbon dioxide into the atmosphere. A modified ethylene forming gene (*efe*) from *Pseudomonas syringae pv. Phaseolicola* was successfully expressed in *Synechocystis* sp. PCC 6803 to produce 3100 μL/L/h of ethylene (Figure 3) [20••].

Fatty acids have also been suggested as precursors for biofuel production. It has been demonstrated that endogenous fatty acyl-ACP synthetase (*slr1609*) overexpression in *Synechocystis* sp. PCC 6803 allows for increased fatty acid activation and improves the yield of fatty alcohol production (Figure 3) [21]. *Synechocystis* sp. PCC 6803 was modified such that it could not only produce but also secrete fatty acids outside the cell [22,21,23•]. *Synechocystis* sp. PCC 6803 was modified to contain fatty acyl-CoA reductase (FAR) genes from jojoba, which catalyze a fatty-acyl-ACP to produce 9.73 μg/OD/L of total fatty alcohols: hexadecanol and octadecanol (Figure 3) [24]. Additionally, this study improved the natural hydrocarbon production by the installment of acetyl-coA carboxylase genes in three strains of cyanobacteria: *Synechocystis* sp. PCC 6803, *S. elongatus* PCC 7942 and *Anabaena* sp. PCC 7120. Eukaryotic algae are also utilized for fatty acid production because they can accumulate lipids up to 70% of their dry biomass [25,26]; however, there are limitations in using algae such as availability of genetic tools due to the complexity of the eukaryotic system [26]. Nevertheless, stress conditions and key enzymes for fatty acid synthesis in the green algae *Haematococcus pluvialis* have been identified [27]. Attempts have also been made to optimize light conditions for fatty acid production in *Nannochloropsis* [28].