Cyanobacterial metabolic engineering for biofuel and chemical production

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Abstract

Rising levels of atmospheric CO₂ are contributing to the global greenhouse effect. Large scale use of atmospheric CO₂ may be a sustainable and renewable means of chemical and liquid fuel production to mitigate global climate change. Photosynthetic organisms are an ideal platform for efficient, natural CO₂ conversion to a broad range of chemicals.

Cyanobacteria are especially attractive for these purposes, due to their genetic malleability and relatively fast growth rate. Recent years have yielded a range of work in the metabolic engineering of cyanobacteria and have led to greater knowledge of the host metabolism. Understanding of endogenous and heterologous carbon regulation mechanisms leads to the expansion of productive capacity and chemical variety. This review discusses the recent progress in metabolic engineering of cyanobacteria for biofuel and bulk chemical production since 2014.

Introduction

Alternatives to fossil fuels have been developed in the past two decades. Biofuel production is a promising solution to oil dependence for energy. Production of biofuels utilizes photosynthetic capture of the energy from sunlight to generate chemical energy in the form of carbon-carbon bonds. Traditional biofuels (e.g. corn ethanol, cellulosic ethanol) are more removed from direct energy and carbon capture, requiring intermediate steps of mechanical refinement and chemical conversion. Conversely, cyanobacteria and other photosynthetic microbes close the gap between photosynthesis and chemical production. Over the last ten years, cyanobacteria have become the new subject of metabolic engineering. Cyanobacteria have a high photosynthetic efficiency (upwards of 10%), significantly better than land plants (~3-4% maximum efficiency) [1,2]. Furthermore, cyanobacteria pose no direct competition for land or resources with agricultural crops [3]. Direct photosynthetic fixation of CO₂ to biofuel and bulk chemicals in cyanobacteria may come to play a significant role in reduction of petroleum dependency and CO₂ emissions. This review discusses the progress in metabolic engineering of cyanobacteria for biofuel and bulk chemical production since 2014 (Table 1).

Alcohols and Diols

2,3-Butanediol

The model cyanobacterium, *Synechococcus elongatus* PCC 7942 (hereafter 7942) has been engineered to produce 2,3-butanediol (23BD) from CO₂ (Fig. 1) [4]. 23BD can

be dehydrated to form the industrial solvent and drop-in fuel additive methylethyl ketone, or to form the polymer precursor 1,3-butadiene. In 23BD biosynthesis, two molecules of pyruvate are condensed to form 2-acetolactate. 2-Acetolactate is decarboxylated to form acetoin, which is reduced to yield 23BD. The 23BD titer was improved from previously observed levels by optimizing the ribosomal binding site sequences preceding each gene in the 23BD pathway [5]. The best strain produced 1.8 fold more 23BD compared to the parent strain.

A common strategy for increasing chemical production is to increase the carbon flux toward the target chemical. This strategy was tested using two strengths of carbon sink pathways to increase intracellular pools of pyruvate, the 23BD precursor [6]. A strong carbon sink pathway overexpressed the three enzymes linking 3-phosphoglycerate (3-PGA) from the Calvin Benson (CB) cycle to pyruvate. A moderate carbon sink pathway overexpressed only the enzyme of the last step (PEP to pyruvate). Expression of the strong sink pathway caused highly growth-deficient cultures under normal and high light (55 and 250 $\mu\text{mol photons/s/m}^2$) conditions. The moderate strength sink increased total carbon yield and specific 23BD production 1.8- and 2.4-fold, respectively. Additionally, although the 23BD pathway was installed under the IPTG inducible promoter P_{LlacO_1} , 23BD production was observed to be similar with and without IPTG [4,7]. Upon further investigation it was discovered that the 23BD pathway genes *alsS* and *adh* contain hidden promoters, which cause constitutive expression [7]. The gene order of the 23BD operon was rearranged to minimize these effects, establishing IPTG-dependent production.

To supplement carbon and reducing equivalents for 23BD production, glucose transporter or xylose transporter and degradation genes were expressed in the 23BD producing strains allowing for sugar consumption [8,9]. In high density cultures ($OD_{730} \sim 5$), final titers from glucose or xylose were increased ten and eight fold, respectively. Under diurnal conditions (12h light:12 h dark) carbohydrate consumption allowed strains to continuously produce 23BD. Under dark conditions, the strains produced 155 mg/L 23BD with glucose and 183 mg/L with xylose in 84 h. Isotopic labelling experiments with ^{13}C -glucose showed that 67% of carbon atoms in 23BD produced by the glucose-consuming strain were derived from glucose. Long term production from the glucose-consuming strain with glucose under continuous light conditions produced 3.0 g/L 23BD in 10 d, with maximum productivity of 22 mg/L/h.

1,3-Propanediol

A 1,3-propanediol biosynthetic pathway was constructed in 7942 [10]. 1,3-Propanediol is primarily used for co-polymer synthesis. The pathway originates with dihydroxyacetone phosphate (DHAP) in the CB cycle (Fig. 1). DHAP is converted to glyceraldehyde-3-phosphate (GAP) by a GAP dehydrogenase, next GAP is reduced to glycerol by a phosphatase. Finally, glycerol is converted to 3-hydroxypropionaldehyde via a coenzyme B12-dependent glycerol dehydratase. Final titer after 14 days was 288 mg/L for an average productivity of 0.9 mg/L/h.

Ethanol

Ethanol production in cyanobacteria was first reported in 1999 [11]. The pathway contains two steps from pyruvate to acetaldehyde by decarboxylation, then reduction of acetaldehyde to ethanol (Fig. 1). Recent *in vitro* kinetic experiments suggested that formation of acetaldehyde was the rate limiting step [12]. Overexpression of the enzyme catalyzing the first step, pyruvate decarboxylase, in *Synechocystis* sp. PCC 6803 (hereafter 6803), resulted in an 83% increase in ethanol production.

Isopropanol

Isopropanol is an important industrial and commercial solvent biosynthesized by a four step pathway. Two acetyl-CoA are condensed to form acetoacetyl-CoA, which is transesterified with acetate to yield acetoacetate and acetyl-CoA. Acetoacetate is reductively decarboxylated to make acetone, then reduced to isopropanol (Fig. 1). It has been shown that chemicals derived from acetyl-CoA require production under dark, anaerobic conditions in cyanobacteria so that glycolytic pathways are activated [13,14]. A five day dark phase was necessary to accumulate acetyl-CoA and acetate, and was followed by a ten day light phase in which precursors were consumed to produce isopropanol. The final titer was 146 mg/L, with average productivity of 0.6 mg/L/h.

Isobutanol

The isobutanol biosynthetic pathway was constructed in 7942 (Fig. 1) [15]. Isobutanol has found wide use as an organic solvent and fuel additive. Ester derivatives of isobutanol are also useful as solvents or polymer plasticizers [16,17]. The native L-

valine biosynthesis intermediate 2-ketoisovalerate (KIV) is decarboxylated to form isobutyraldehyde, then reduced to isobutanol [15]. A glycogen synthase mutant ($\Delta glgC$) of 7942 was constructed to increase carbon flux to isobutanol [18]. Deletion of *glgC* caused growth inhibition under high light without the isobutanol pathway. Introduction of the isobutanol pathway provided a sink for excess energy and carbon, and carbon partitioning to isobutanol was enhanced from 22% to 52%.

Hydrocarbons

Ethylene

Ethylene is an important component of many polymers. Ethylene production has been engineered in 7942 [19] and 6803 [20] (Fig. 1). Supplementing photosynthetic production with exogenous sugars to improve ethylene titers was recently explored [21]. Expression of *E. coli* genes encoding xylose isomerase and xylulokinase enable 6803 to utilize xylose in addition to CO₂ [21]. Sugar feeding improved ethylene production up to 64% (11 mg/L in 12 hours).

Fatty carbohydrates

Fatty Acids

Fatty acids are an attractive target for biosynthesis because they are easily converted to viable diesel-like compounds. In lipid synthesis, hydrocarbon chains are elongated by the condensation of acyl-acyl carrier protein (ACP) with CoA-bound

carbon units. Cleavage of acyl-ACP by a thioesterase yields free fatty acids (FFA) (Fig. 1). Deletion of acyl-ACP synthetase/long-chain-fatty acid CoA ligase prevents recycling of FFA and allows for their accumulation in microbial hosts. 6803, 7942, and *Synechococcus* sp. PCC 7002 (hereafter 7002) have been engineered for fatty acid production [22-24]. Optimization of FFA production in 6803 was achieved by removing alternative carbon sinks and increasing flux to fatty acid biosynthesis produced a titer and productivity of 197 mg/L and 0.4 mg/L/h, respectively [22]. Similar optimization in 7942 produced a titer and productivity of 49 mg/L and 0.1 mg/L/h [23]. In 7002, FFA was further optimized by overexpression of heterologous RuBisCO, and resulted in a titer and productivity of 131 mg/L and 0.3 mg/L/h, respectively [24].

Fatty alcohols

Fatty alcohols are energy rich molecules that can readily be used as fuel alternatives or converted to fatty esters to form waxes. They can be derived from reduction of thioester intermediates such as acyl-ACP or acyl-CoA (Fig. 1). Prior work with 6803 produced up to 10 µg/OD₇₃₀/L of fatty alcohol by expression of a fatty acyl-CoA reductase from *Simmondsia chinensis* [25]. Additionally, preventing carbon partitioning to glycogen and poly-3-hydroxybutyrate (poly-3HB) improved fatty alcohol titer to 0.8 mg/g dry cell weight (DCW) [26]. By expressing a fatty aldehyde reductase from *Marinobacter aquaeolei* VT8, coupled with the deletion of reductases from native alkane formation pathways (fatty acyl-ACP reductase (*sll0209*) and fatty aldehyde reductase (*sll0208*)), 3 mg/g DCW of fatty alcohol was produced from 6803 [27].

Fatty Hydrocarbons

As indicated above, alkanes can be synthesized from fatty acid or fatty aldehyde substrates by subsequent action of an acyl-ACP reductase (AAR) and an aldehyde deformylating oxygenase (ADO), respectively (Fig. 1) [28]. Expression of *ado* and *aar* genes in the α -olefin producing *Synechococcus* sp. NKBG15041c produced 4 μ g/g DCW of heptadecane as well as small amounts of unsaturated hydrocarbons, including 1,14-nonadecadiene and 1-nonadecene [29].

Carbohydrates

Glycogen

Glycogen is a promising feedstock for biofuel production, and is naturally accumulated in cyanobacteria for carbon storage. Cyanobacteria typically accumulate glycogen in response to nitrogen-depletion by a two-step pathway in which glucose-1-phosphate is converted to glycogen by an ADP glucose pyrophosphorylase (GlgC) and a glycogen synthase (GlgA) (Fig. 1). 7002 was studied for its ability to accumulate glycogen without any strain engineering, in a range of conditions including light intensity, CO₂ concentration, and salinity [30]. Glycogen was maximally produced at a titer and productivity of 3.5 g/L and 0.5 g/L/d, respectively, in conditions of high light intensity (600 μ mol photons/m²/s), high CO₂ concentration (2%), nitrogen depletion (13-15 mM nitrate), and moderate salinity (brackish water).

Mannitol

D-Mannitol is widely used in the medical, pharmaceutical, chemical, and food industries. 7002 was engineered to produce mannitol solely from CO₂ by overexpression of two enzymes: Mannitol-1-phosphate dehydrogenase (MtlD) from *E. coli*, and mannitol-1-phosphatase (Mlp) from the protozoan chicken parasite *Eimeria tenella* (Fig. 1) [31]. To increase mannitol yield further, the glycogen storage pathway, which competes for F6P, was deleted. Maximal titer and productivity of the engineered strain were 1.1 g/L and 0.15 g/L/d, respectively.

Lactate

L-Lactic acid is a commodity chemical used in the food and pharmaceutical industries, and to produce biodegradable polymers. It can be produced from the central metabolite pyruvate by a single enzymatic step catalyzed by an NADH-dependent lactate dehydrogenase (Ldh) from *Lactococcus lactis* (Fig. 1). L-Lactic acid production from 6803 has been previously demonstrated [32]. It was recently improved by combining four strategies: increased *ldh* gene dosage, use of an Ldh from *Bacillus subtilis* mutated for use of NADPH, inactivation of the enzymatic reaction that competes for the pyruvate precursor PEP, and by overexpression of the enzyme that generates pyruvate (pyruvate kinase from *Enterococcus faecalis*) [33]. This combination in 6803 achieved a titer of 840 mg/L, and productivity of 22 mg/g DCW/h. It was demonstrated

that under nitrogen-limited conditions, deletion of glycogen storage increased L-lactate production two-fold, but poly-3HB deletion had no effect [34].

The enantiomer D-lactate is valuable as a precursor of thermostable polylactide, which is used in the manufacture of biodegradable plastics and artificial biological tissues. Several organisms, including 6803, harbor a D-lactate dehydrogenase (D-Ldh)[35]. Recently, expression of native and other heterologous D-Ldhs in 6803 with inactivated poly-3HB and acetate synthesis pathways was shown to produce 0.4 g/L of D-lactate using its native D-Ldh, and 1.1 g/L D-lactate using the D-Ldh from *Lactobacillus delbrueckii* [35].

Glucosylglycerol

Glycosylglycerol (GG) has protein stabilizing and antioxidant properties. 6803 was engineered for GG production by deletion of several genes: Those encoding the GG and other osmoprotectant uptake transporters GgtC and GgtD, and the GG biosynthesis repressor GgpR [36]. Gel-encapsulation of engineered 6803 cells increased GG production to maximal titers of 1.6 g/L [36].

Carboxylic acid

3-hydroxypropionic acid

3-hydroxypropionic acid (3HP) is a biodegradable polymer precursor. Two 3HP production pathways have been constructed in 7942 (Fig. 1) [37]. In the malonyl-CoA dependent pathway, malonyl-CoA is reduced to 3HP via malonate semialdehyde. The

alternative β -alanine pathway begins with carboxylation of PEP to form oxaloacetate. Subsequent transamination yields aspartate, followed with decarboxylation to β -alanine. transamination of β -alanine converges with the former pathway in malonate semialdehyde to be reduced to 3-HP. These two pathways were expressed concomitantly in 7942 [37]. Independently, the malonyl-CoA and β -alanine pathways produced 659 mg/L (maximum productivity; 98 mg/L/d) and 186mg/L (maximum productivity; 25 mg/L/d) 3-HP in 16 d, respectively. The strain with both pathways produced 665 mg/L (maximum productivity; 102 mg/L/d) 3HP in 16 d.

3HP production was also attempted in 6803. In the engineered pathway, malonyl-CoA is doubly reduced to 3-HP by a single enzyme, malonyl-CoA reductase from *Chloroflexus aurantiacus* [38]. The engineered strain produced 689 mg/L 3HP in 6 d. Production was improved by overexpression of endogenous acetyl-CoA carboxylase, biotinylase, and pyridine nucleotide transhydrogenase genes, the latter to improve NADH/NADPH ratios perturbed by malonyl-CoA reductase activity. Assembly of all modifications described above produced 837 mg/L 3HP in 6 d.

poly-3-hydroxybutyrate-co-4-hydroxybutyrate

As with 3HP, isomers of HB are biodegradable polymer precursors. The copolymer poly-3-hydroxybutyrate-co-4-hydroxybutyrate (P3HB4HB) is desirable for its versatile, tunable properties depending upon its composition [39,40]. In 7002, expression of a heterologous poly-3HB synthesis operon (*phaABEC* from *Chlorogleopsis fritschii* PCC 9212) converges with an engineered 4HB-CoA pathway to make P3HB4HB (Fig. 1) [41]. 3HB-CoA and 4HB-CoA are condensed into copolymer by polyhydroxyalkanoate

synthase encoded by *phaEC*. The best production was ~4.5% of total DCW with 12% 4HB composition.

Terpenes and Isoprenes

Most engineered terpene and isoprenoid production in cyanobacteria is drawn from the native terpene production pathway, the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway [42-44]. Generally, isoprenoid compounds enjoy broad application in industrial and consumer goods because of a wide range of molecular weights and structural diversity. Molecules in the range of monoterpene (C₁₀) to diterpene (C₂₀) compounds have been identified as good replacements for petroleum-based jet-fuel (i.e. kerosene or paraffin).

Isoprene

Isoprene is a common feedstock chemical for manufacture of rubbers, adhesives, flavor/fragrances, and fuels. Isoprene production in 6803 was improved by expressing exogenous, prokaryotic mevalonic acid (MVA) pathway genes (Fig. 1) [45]. Expression of the MVA pathway circumnavigates the endogenous MEP pathway regulation of 6803, forming a parallel route to dimethylallyl diphosphate. Compared with a strain expressing only the isoprene synthase gene, the addition of the MVA pathway produced ~2.4 times as much isoprene. The final titer was 320 µg/L after 196 h. Growth conditions inhospitable to intolerant predatory microbes has been studied to demonstrate the amenability of cyanobacteria to scaled-up isoprene production [46]. It was shown that

isoprene titer and 6803 growth were unaffected by alkaline (up to pH 10) or high salinity (up to 200mM NaCl) conditions, even in a closed reactor.

Limonene

Limonene, a cyclic monoterpene found in the rind of citrus fruit, has numerous commercial applications, including flavoring and pharmaceuticals. It has gained the greatest attention as a metabolic product for its potential as a candidate in jet fuel blending [47,48]. Limonene was successfully produced from the filamentous, nitrogen fixing cyanobacterium *Anabaena* sp. PCC 7120 (hereafter 7120) (Fig. 1) [49]. A maximum productivity of 3.6 µg/L/OD₇₀₀/h and titer of 0.5 mg/L over 12 d was achieved by overexpression of three key enzymes (i.e. 1-deoxy-D-xylulos in endogenous terpene synthesis pathway and the limonene synthase gene from *Picea sitchensis*. Limonene has also been produced from 6803 (56 µg/L/d) by overexpression of the limonene synthase gene from *Schizonepeta tenuifolia* and three endogenous terpene biosynthesis genes [50].

Farnesene

The sesquiterpene farnesene has commercial applications in the production of lubricants, cosmetics, fragrances, and as a biofuel. Biological production of farnesene has been established in *Saccharomyces cerevisiae* on an industrial scale by Amyris Biotechnologies. However, the necessity of providing sugar as a carbon feedstock adds extra costs to this production strategy. Engineered farnesene production was

established in 7120 via expression of the farnesene synthase gene from *Picea abies* [51] to achieve maximum productivity of 69 µg/L/OD₇₀₀/d and a titer of 305 µg/L after 15 d (Fig. 1).

Bisabolene

Bisabolene has key commercial applications in biofuels, bioplastics, pharmaceuticals, nutraceuticals, and cosmetics. Engineered 7002 produced limonene and bisabolene via overexpression of the L-limonene synthase gene from *Mentha spicata* and (E)-α-bisabolene synthase gene from *Abies grandis* (Fig. 1) [52]. Titters reached 4 mg/L limonene and 0.6 mg/L α-bisabolene. Maximum productivities were 50 µg/L/h for limonene and 6 µg/L/h for bisabolene.

Squalene

Squalene is a 30-carbon isoprenoid that is currently used commercially in the production of cosmetics and vaccines. If it could be produced on a larger scale, it would be an excellent candidate biofuel feedstock. Squalene is naturally produced by a number of bacteria as an intermediate in the biosynthesis of hopene, a precursor in hopanoid biosynthesis. Squalene-hopene cyclase, the enzyme responsible for the conversion of squalene to hopene, has been characterized in a number of organisms. Genes for both squalene synthase (*sqs*) and squalene-hopene synthase (*shc*) were characterized in 6803 (Fig. 1) [53]. Deletion of *shc* allowed squalene accumulation to 0.7 mg/OD₇₅₀/L.

306

307 **Manoyl Oxide**

308 The diterpenoid forskolin from *Coleus forskohlii* is a natural product of great interest
309 to the pharmaceutical industry. It has been shown to modulate cAMP levels in
310 mammalian cells and is currently used in the treatment of glaucoma. Using 6803, a
311 maximum titer of 0.5 mg/g DCW of the forskolin precursor (13R)-manoyl oxide [54] was
312 achieved (Fig. 1).

313

314 **Caffeic acid**

315 Caffeic acid is a natural phenylpropanoid produced by various plant species, and
316 exhibits anticancer activity. Caffeic acid is produced from either tyrosine or
317 phenylalanine through the *p*-coumaric acid (Fig. 1). A P450-dependent
318 monooxygenase, *p*-coumarate-3-hydrolase (C3H) catalyzes the NADPH-dependent
319 formation of caffeic acid [55]. Heterologous expression of a codon-optimized C3H
320 encoding gene *ref8* from *Arabidopsis thaliana* in 6803 produced 7 mg/L of caffeic acid
321 [56]. This study shows that cyanobacteria may serve as P450-expression hosts suitable
322 for industrial production.

323

324 **Conclusions**

325 Progress with engineered strains of cyanobacteria for biofuel and various industrially
326 relevant chemicals is encouraging. Chief amongst design principles is balancing flux

through a pathway by considering kinetic efficiency of the pathway overall [57]. To remove bottlenecks, enzyme expression levels, availability of precursor metabolites, and cofactor balance within the whole cell must be optimized. Commercial production will require higher titers and productivities. Although a principal design element of metabolic engineering is the incorporation of exogenous genetic elements, the breadth of molecular complexity and diversity is growing in direct proportion to our understanding of inherent metabolic pathways. Understanding host metabolism and further development of synthetic biology tools will play a major role in ultimate success [58-62]. For example, alternatives to strict photoautotrophy take advantage of the adaptability of photosynthetic mechanisms as a virtually unlimited source of reductive equivalents [8,9]. By installing the machinery for non-photosynthetic carbon intake, flux is increased through central metabolism, bolstering pools of precursor metabolites. Ideally, there is sufficient carbon intake to sustain high-efficiency chemical production and basal cellular metabolism. Even under low-light or dark conditions, limitations in ATP and reductive cofactors (i.e. NAD(P)H) are superceded. Thus, a new cyanobacterial paradigm of photo-heterotrophy could make industrial-level productivity an attainable goal.

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***5. Oliver JW, Machado IM, Yoneda H, Atsumi S.**

Production of 23BD in 7942 was improved by altering the ribosomal binding site. They utilized a combinatorial approach with 4 different RBS sequences. A broad range in 23BD production from 207 mg/L to 496 mg/L was observed. This demonstrates the efficacy of this approach and the importance of regulating gene expression for achieving high titer production.

***7. Nozzi NE, Atsumi S.**

The efficacy of the promoter P_{LacO₁} was investigated and strong control of gene expression was demonstrated. Constitutive promoters in two of the genes in the 23BD pathway (*alsS* and *adh*) were discovered and gene order was shown to be critical.

****9. McEwen JT, Kanno M, Atsumi S.**

Previous work had identified strategies for sugar utilization in 7942. This work was further expanded upon by demonstrating 23BD production from engineered 7942 strains under various light conditions (light, dark, and diurnal) with glucose or xylose. From both the glucose (*galP*) and xylose (*xylEAB*) strains, a significant increase in both biomass accumulation, and 23BDO production was observed.

****10. Hirokawa Y, Maki Y, Tatsuke T, Hanai T.**

A biosynthetic pathway of 1,3-propanediol was established in 7942. The addition of a glycerol dehydratase allowed for the conversion of glycerol to 1,3-propanediol. The engineered strain produced 288 mg/L after 14 days under continuous light conditions.

***21. Lee T-C, Xiong W, Paddock T, Carrieri D, Chang F, Chiu H-F, Ungerer J, Juo S-HH, Maness P-C, Yu J:**

Two genes from *E. coli* (*xylAB*) were installed into 6803 to utilize xylose. The addition of xylose consumption genes and xylose improved ethylene production by 64%.

***24. Ruffing AM.**

Free fatty acid production had been previously demonstrated in two cyanobacterial species (7942 and 6803). Although previous studies had reported some negative physiological effects, similar titers (>130mg/L) were demonstrated in 7002 without any negative physiological effects.

***33. Angermayr SA, van der Woude AD, Correddu D, Vreugdenhil A, Verrone V, Hellingwerf KJ.**

Lactic acid production in 6803 was improved by optimizing gene expression, increasing carbon flux and eliminating competing pathways. They were further able to increase production by optimizing Ldh through site directed mutagenesis. Carbon partitioning to lactic acid was increased from 5% to over 50%.

***37. Lan EI, Chuang DS, Shen CR, Lee AM, Ro SY, Liao JC**

Two different routes to produce 3-HP in 7942 were explored. The malonyl-CoA dependent pathway produced 3HP at 665 mg/L. The β -alanine dependent pathway achieved 186 mg/L 3HP.

***41. Zhang S, Liu Y, Bryant DA**

A pathway from *C. fritschii* PCC 9212 was introduced into 7002 to produce P3HB4HB. 3HB-CoA and 4HB-CoA are condensed into copolymer by polyhydroxyalkanoate synthase. The production was 4.5% of total DCW with 12% 4HB composition.

***45. Bentley FK, Zurbriggen A, Melis A**

Isoprene production in cyanobacteria had been previously demonstrated with limited productivities. Isoprene production was improved by increasing partitioning to production by overexpression of the mevalonic acid pathway genes, resulted in 2.5 fold increase.

****52. Davies FK, Work VH, Beliaev AS, Posewitz MC**

The terpenoids limonene and α -bisabolene were produced in 7002 through the expression of either limonene synthase or α -bisabolene synthase. Final titers of limonene and α -bisabolene reached 4 mg/L and 0.6 mg/L respectively.

****54. Englund E, Andersen-Ranberg J, Miao R, Hamberger B, Lindberg P.**

Forskolin is a diterpenoid with significant pharmaceutical applications. Current production methods require plant extractions. Through the addition of two diterpenen

597 synthases the engineer 6803 strain produced the precursor to forskolin, 13R-manoyl
598 oxide, at 0.45 mg/g DCW.

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Fig. 1 Various chemicals produced from cyanobacteria.

Chemicals in red are discussed in this review. CB, Calvin Benson; TCA, tricarboxylic acid, 3PGA; 3-phosphoglycerate, DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; 23BDO, 2,3-butanediol; 13PDO, 1,3-propanediol; 3HP, 3-hydroxypropionic acid; 3HB, 3-hydroxybutyrate; 4HB, 4-hydroxybutyrate.

607 Table 1 Chemical production in cyanobacteria

product	titer	productivity	host	non-CO ₂ carbon	reference
23BDO	3.0 g/L	13 mg/L/h	7942	glucose	[9]
13PDO	0.3 g/L	0.9 mg/L/h	7942		[10]
Ethanol	2.3 g/L	11 mg/L/h	6803		[12]
Isopropanol	0.2 g/L	0.6 mg/L/h	7942		[14]
Isobutanol	0.6 g/L	2.9 mg/L/h	7942		[18]
Fatty alcohols	2.9 g/g DCW	21 mg/g DCW/h	6803	xylose	[27]
Ethylene	11 mg/L	0.9 mg/L/h	6803		[21]
Fatty acids	130 mg/L	0.3 mg/L/h	7002		[24]
Alkanes	4.2 g/g DCW		NKBG15041c		[29]
Glycogen	3.5 g/L	21 mg/L/h	7002		[30]
Mannitol	1.1 g/L	3.8 mg/L/h	7002		[31]
L-Lactate	0.8 g/L	2.5 mg/L/h	6803		[33]
D-Lactate	1.1 g/L	11 mg/L/h	6803		[35]
GG	1.6 g/L	2.1 mg/L/h	6803		[36]
3HP	0.7 g/L	1.7 mg/L/h	7942		[37]
3HP	0.8 g/L	5.8 mg/L/h	6803		[38]
P3HB	4% DCW		7002		[41]
P3HB4HB	4.5% DCW		7002		[41]
Isoprene	320 µg/L	1.6 µg/L/h	6803		[45]
Limonene	4 mg/L	40 µg/L/h	7002		[52]
Farnesene	310 µg/L	850 ng/L/h	7120		[51]
Bisabolene	700 µg/L	7 µg/L/h	7002		[52]
squalene	670 µg/L		6803		[53]
13R-manoyl oxide	0.5 mg/g DCW	4.7 µg/L/h	6803		[54]
Caffeic acid	0.7 mg/L	10 µg/L/h	6803	p-coumarate	[56]

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