UC Berkeley
UC Berkeley Previously Published Works

Title

Engineering isoprene synthesis in cyanobacteria

Permalink
https://escholarship.org/uc/item/2hs0w919

Journal
FEBS Letters, 592(12)

ISSN
0014-5793

Authors
Chaves, Julie E
Melis, Anastasios

Publication Date
2018-06-01

DOI
10.1002/1873-3468.13052

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

FEBS Letters

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

Engineering isoprene synthesis in cyanobacteria

Julie E. Chaves and Anastasios Melis

Plant and Microbial Biology, University of California, Berkeley, CA, USA

Correspondence
A. Melis, Plant and Microbial Biology, University of California, Berkeley, 111 Koshland Hall, MC-3102, CA 94720-3102, USA
Fax: +1 510-642-4995
Tel: +1 510-642-8166
E-mail: melis@berkeley.edu

(Received 26 February 2018, revised 21 March 2018, accepted 5 April 2018, available online 27 April 2018)

doi:10.1002/1873-3468.13052

Edited by Wilhelm Just

The renewable production of isoprene (Isp) hydrocarbons, to serve as fuel and synthetic chemistry feedstock, has attracted interest in the field recently. Isp (C_5H_8) is naturally produced from sunlight, CO_2 and H_2O photosynthetically in terrestrial plant chloroplasts *via* the terpenoid biosynthetic pathway and emitted in the atmosphere as a response to heat stress. Efforts to institute a high capacity continuous and renewable process have included heterologous expression of the Isp synthesis pathway in photosynthetic microorganisms. This review examines the premise and promise emanating from this relatively new research effort. Also examined are the metabolic engineering approaches applied in the quest of renewable Isp hydrocarbons production, the progress achieved so far, and barriers encountered along the way.

Keywords: bioenergy; dimethylallyl diphosphate; isopentenyl diphosphate; isoprene; metabolic engineering; *Synechocystis*; synthetic biology; terpenoids

Nearly, all contemporary industrial products are generated from petroleum and petroleum-derived feedstocks including fuels, plastics, adhesives, lubricants, solvents, rubber, cosmetics, and pharmaceuticals. The overuse of fossil resources for the generation of ubiquitous products has led to major environmental issues including the atmospheric buildup of CO_2, diminishing of the ozone layer, pollution, and climate change. It has been projected that the global energy demand will increase 50–60% by 2030 due to the increase in population size and higher standards of living [1,2]. Thus, it is essential that renewable and environmentally friendly technologies are developed to supplement and gradually replace dependence on fossil sources.

Isoprene (Isp) is one such industrially important chemical, as one million tons of it are produced annually from petrochemicals and consumed for industrial applications on a global scale [3]. In addition to its fossil origin, Isp is a naturally produced chemical, emitted from the leaves of many deciduous and evergreen plants including eucalyptus, oak, poplar, and some legumes in response to heat and drought stress [4–7]. Emission of Isp in response to heat stress from terrestrial plants is thought to confer thermotolerance, viewed as a short-term response (minutes-to-hours) to this abiotic stress. The yearly generation of Isp emissions by land plants was estimated to be about 600 million metric tonnes [8], with about half of that produced by tropical broadleaf trees, and the remainder originating from the photosynthesis of shrubs. However, collecting for industrial applications such enormous amounts of Isp is impractical. On the other hand, aquatic photosynthetic organisms, including microalgae and cyanobacteria, are not known to possess the Isp synthase (IspS) gene and have not been systematically tested for the Isp production process [9,10].

The majority of industrially produced Isp is utilized in the production of elastomers (~ 95%), and a small fraction is used as feedstock in the synthetic chemistry

Abbreviations
cpcB*Isps, isoprene synthase fusion to the cpcB gene; cpcB, first gene in the cpc operon encoding the β-phycocyanin subunit; cpc, operon encoding the phycocyanin subunits and associated linker polypeptides; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; Isp, isoprene; IspS, isoprene synthase; MEP, 2-C-methyl-D-erythritol-4-phosphate; RuBisCO, ribulose bis-phosphate carboxylase-oxygenase.

industry [3]. About 60% of total Isp used for commercial rubber production is generated from fossil fuels, and about 40% is derived from natural resources, primarily from the sap of the tropical rubber tree *Havea brasiliensis* [11], which is collected in the form of natural rubber. This tree requires temperatures of 25–30 °C, a minimum of 2000 mm of water from rainfall per year, and high humidity, thus its growth and exploitation is limited to the tropic regions of the earth [12–14].

Cyanobacteria are attractive microorganisms to use as a chassis for the photosynthetic generation of Isp. Cyanobacteria are primordial oxygenic photosynthetic prokaryotes, and the ancestor of the plant and green microalgal chloroplasts [15]. Using only sunlight, CO_2, water, and minimal nutrients, cyanobacteria are able to produce biomass and chemicals more efficiently than land plants, thereby helping to achieve the theoretical solar-to-biomass energy conversion efficiency maximum of about 8–10% [15,16], relative to only about 2%, or less [17,18] achieved by Isp-producing land plants. Growth rates of cyanobacteria are much faster than those of plants, and the genetic tractability of cyanobacteria is much greater than in algae. In addition, cyanobacteria, as unicellular microorganisms, do not need to invest photosynthetic resources in root, stem, and leaf tissues, hence they can be programmed to invest a greater proportion of their photosynthate to heterologous product generation, for example, Isp. This can be achieved upon growth in fully enclosed outdoor photobioreactors, under a wide range of conditions, on nonarable land, and with fresh, brackish, or seawater so as not to compete with the cultivation of crop plants [16].

In 2010, the first report of cyanobacterial production of Isp was published [19] *via* the heterologous expression of the IspS gene from kudzu (*Pueraria montana*) in the model cyanobacterium *Synechocystis*. Since then, several modifications and improvements have been introduced to optimize this technology for the future commercial production of Isp from cyanobacteria cells.

Reactants and reactions

Isoprene is one derivative among the largest class of naturally produced chemicals, the isoprenoids, or terpenoids. There are over 80 000 different known terpenoids, with the number of defined structures doubling every decade since the 1970s [20,21]. Terpenoids are functionally important in the living cell, as they provide for membrane fluidity, protein prenylation, electron transport molecules, steroid backbones, fragrances for pollinator attraction, and toxins for predator and pathogen defense [22,23]. In photosynthetic systems, the terpenoid biosynthetic pathway yields essential compounds such as carotenoids, the phytol tail of chlorophyll, and quinone prenyl tails, all of which are essential for functional photosynthesis.

The universal precursors for terpenoid biosynthesis in all living systems are the 5-carbon prenyl diphosphates dimethylallyl diphosphate (DMAPP) and its isomeric form isopentenyl diphosphate (IPP) [9,24]. DMAPP serves as a primer for the sequential addition of IPP molecules in a multitude of different numbers to form the hydrocarbon backbone of terpenoids. Most terpenoids are then further functionalized from these backbones through ring closures, benzoylation, acetylation, hydroxylation, and glycosylation [23].

It is of interest and uncommon in the annals of evolution that two completely different biosynthetic pathways function in nature to generate DMAPP and IPP. The mevalonic acid (MVA) pathway, and the methylerythritol phosphate (MEP) pathway [25–27] (Fig 1.). The MVA pathway was the first pathway defined, and is found mainly in eukaryotes and in archaea [23], as well as in a few eubacteria, and the cytosol and mitochondria of plants and fungi [28]. Acetyl-CoA is the feeder molecule for the MVA pathway, which is then transformed into IPP through six enzymatic reaction steps. In this pathway, DMAPP must be generated through the isomerization of IPP *via* the Fni IPP isomerase (Ipi) [21–23]. In cyanobacteria, microalgae, and plant chloroplasts, IPP and DMAPP are generated through the MEP pathway from glyceraldehyde-3-phosphate (G3P) and pyruvate (Pyr), as the feeder molecules, by the action of a set of seven consecutive enzymatic reactions [29], with the final enzyme, 4-hydroxy-3-methyl-2-(E)-butenyl-diphosphate (HMBPP) reductase (encoded by the *IspH* gene) generating both DMAPP and IPP from the same reactant. During photoautotrophic growth of cyanobacteria, the primary MEP feeder molecules, G3P and Pyr, are in higher abundance than that of Acetyl-CoA [30]. The MEP pathway requires six starting molecules of CO_2 to be converted to G3P and Pyr through the Calvin Benson Cycle, whereas the MVA pathway requires three acetyl-CoA as the initial reactants. Each process entails a loss of CO_2 in the process of IPP and/or DMAPP synthesis [30]. Acetyl-CoA generating pathways such as glycolysis and the pentose phosphate pathway are generally less active during cyanobacterial photosynthesis with respect to heterotrophic organisms [31], functioning mainly in the building of carbon precursors for cellular structures [32]. Carbon flux through acetyl-CoA is greater during

heterotrophic growth, such as during glycolysis, dark fermentation or oxidative respiration of glycogen or starch stores [15,33].

In the cyanobacterial MEP pathway, DMAPP and IPP are initially generated simultaneously from HMBPP in a ratio of DMAPP : IPP = ~ 1 : 6, followed by isomerization *via* the Ipi to increase this ratio to ~ 1 : 3 [34] (Fig. 1). Primary isoprenoid synthesis occurs upon head-to-tail additions of one or several IPPs to DMAPP [24], yielding molecules with 10, 15, 20, or > 40 carbon structures. C30 triterpenes and C40 tetraterpenes are generated by tail-to-tail fusion of two C15 sesquiterpenes and C20 diterpenes, respectively. Therefore, a high IPP-to-DMAPP steady-state ratio in the cell makes biochemical sense, as there is a greater requirement for IPP than for DMAPP for the synthesis of the vast majority of isoprenoid products. However, the IspS enzyme utilizes only DMAPP as the substrate for Isp production. Hence, the small DMAPP pool size is a limiting parameter in the effort to achieve a high capacity cyanobacterial Isp production. Moreover, the total carbon flux through the MEP pathway is only about 5% of the total carbon sequestered through photosynthesis [35], thus only about 1.66% of the total carbon that enters the photosynthetic Calvin-Benson Cycle will become DMAPP, and an even smaller fraction can become Isp after investment of DMAPP and IPP in the endogenous cellular terpenoids (Fig. 2).

Cofactor and energy carrier components are also essential pieces of the enzymatic catalysis of Isp production. In the light reactions of photosynthesis, ATP and NADPH are generated in a specific ratio, which may impact the efficiency of the isoprenoid biosynthetic pathway [15,36]. During the course of photosynthesis, ATP and NADPH are generated at a ratio of ~ 1.5 : 1 depending on the state of the Q-cycle [36,37]. The stoichiometric requirement of energy molecules (ATP/NADPH) through the isoprenoid biosynthetic pathway has been calculated to fall within this range at 1.35 : 1 [36,38], thus Isp production should not be hindered by the natural cyanobacterial pool size of ATP and reductant availability.

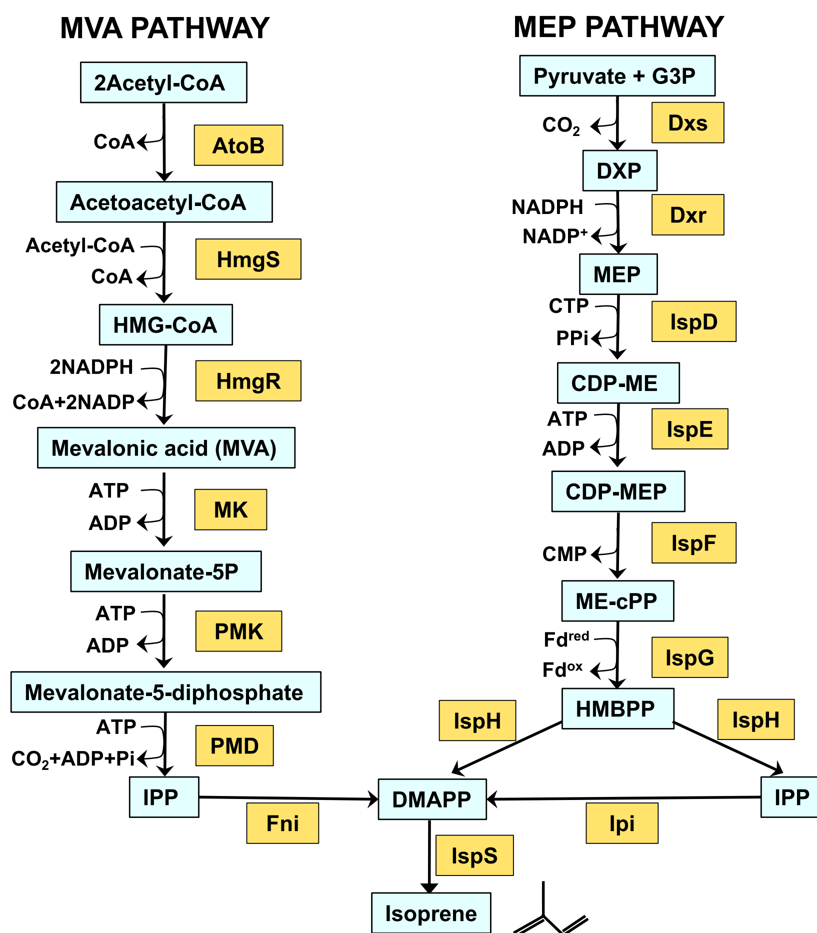

Fig. 1. Comparative endogenous substrate flux to IPP and DMAPP through the MVA and MEP biosynthetic pathways. Acetyl-CoA and pyruvate-G3P are the primary substrates for the MVA and MEP pathways, respectively. In both cases, IPP and DMAPP are the end products. (Left) MVA pathway enzymes: AtoB, acetyl-CoA acetyl transferase; HmgS, Hmg-CoA synthase; HmgR, Hmg-CoA reductase; MK, mevalonic acid kinase; PMK, mevalonic acid 5-phosphate kinase; PMD, mevalonic acid 5-diphoshate decarboxylase; Fni, IPP isomerase. (Right) MEP pathway enzymes: Dxs, deoxyxylulose 5-phosphate synthase; Dxr, deoxyxylulose 5-phosphate reductoisomerase; IspD, diphosphocytidylyl methylerythritol synthase; IspE, diphosphocytidylyl methylerythritol kinase; IspF, methyl erythritol-2,4-cyclodiphosphate synthase; IspG, hydroxymethylbutenyl diphosphate synthase; IspH, hydroxymethylbutenyl diphosphate reductase. Schematic adapted from [29].

Barriers to high capacity production

There are several factors to consider in the scalability of cyanobacterial production of Isp including: strain selection, growth medium, culture conditions, and the light regime. There are also specific process optimization issues that need to be addressed. Cyanobacteria do not naturally produce Isp and, so far, heterologous transformation to produce Isp has been applied only to the unicellular strains *Synechocystis* sp. PCC 6803 [19,29,39,40] and *Synechococcus elongatus* [30]. In both microorganisms, Isp, being a volatile and highly hydrophobic hydrocarbon, spontaneously separated upon diffusion from the cells and the aqueous phase of the medium. In fully enclosed reactors, it accumulated in the gaseous phase above the liquid culture. Such spontaneous separation alleviated a need for culture dewatering and biomass harvesting for product extraction, a difficult feat as unicellular cyanobacteria are very small, typically ranging in size from 0.3 to 0.5 μm [41]. However, cyanobacteria biomass has value, as there are other products that may be useful for pharmaceutical purposes such as polyketides, peptides, alkaloids, and other larger size terpenes [42,43]. In addition, the biomass could be used for hydrothermal liquefaction generation of bio-oil [44,45], or as carbohydrate feedstock for fermentation derived processes [46,47]. Utilizing as much of the biomass for as many markets as possible would make the process more economically viable. Choosing a filamentous strain with a larger size (~ 200 μm) would make separation of the biomass from the growth media through filtration and the natural formation of cell aggregates easier. Well known genera of the filamentous phenotype include: *Anabaena* [48–50], *Nostoc* [51], and the most studied in outdoor cultivations, *Spirulina* [52–54]. Alternatively, engineering approaches to change the morphology of the unicellular *Synechococcus* sp PCC 7942 have also been shown to successfully increase the size of the cell, resulting in enhanced sedimentation and easier biomass recovery [55]. Strain selection can also be optimized at the ecological level, using mixed-species cultures to promote culture stability and productivity. Polyculture assemblages have been proposed to potentially outperform monocultures [56,57]. Furthermore, investigation of Isp production using these strains and cultivation practices that employ nutrients from wastewater could be advantageous to high capacity production [58].

Cyanobacteria are a very diverse group found among a range of ecotypes, including extremophile environments [59–61]. The ideal commercial strain for Isp production would be thermophilic, halophilic, and alkalophilic. The IspS is a protein typically upregulated in response to heat stress [4], and has been

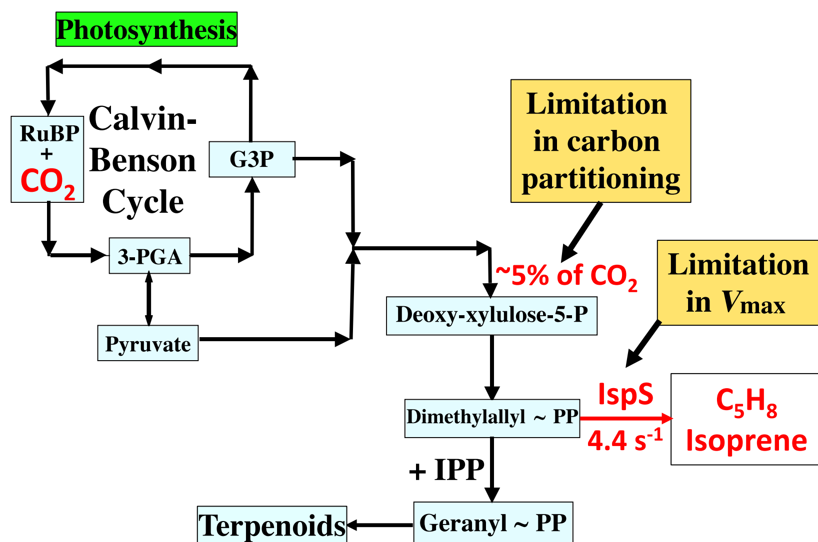

Fig. 2. Metabolic pathway linking photosynthesis in the Calvin Benson Cycle to the MEP pathway and terpenoid and Isp production in cyanobacteria, as discussed in this Review. In the vast majority of photosynthetic systems, carbon partitioning is primarily directed toward sugar synthesis leading to biomass accumulation, with the terpenoid biosynthetic pathway consuming a minor 5% aliquot of the photosynthetically converted CO_2 substrate. Barriers to a high capacity photosynthetic Isp production process in cyanobacteria include the limited endogenous carbon partitioning to the terpenoid biosynthetic pathway, and the relatively slow turnover (slow V_{max}) of the IspS itself, both of which limit the overall rate and yield of Isp production. PGA, phosphoglyceric acid; RuBP, ribulose bisphosphate.

shown to be most active at 40 °C under *in-vitro* conditions [58]. In addition to the activity of the IspS, a thermophilic phenotype would be essential for growth in bioreactors on nonarable soil with high solar exposure such as the desert. Using a strain that can be grown at high salinity would gain the ability to utilize seawater or brackish water instead of fresh water, and alkaline tolerance would provide another parameter to screen out potential contaminants. Isp generation has been shown in the fresh water strain *Synechocystis* sp. PCC 6803 grown in seawater concentrations of 3.5% w : v NaCl [40,62] up to pH 10 [62], showing the natural plasticity of the strain to tolerate a range of environmental conditions, while generating the product of interest. Recently, a *S. elongatus* sp PCC 7942 strain was developed to tolerate higher stress conditions for outdoor cultivation by overexpression of the *hspA* gene [63]. This modification resulted in an enhanced ability to tolerate temperatures up to 45 °C, high sunlight intensity, and seawater concentrations of NaCl (3.5%).

The nutrient composition of the growth media is also an essential aspect of scale up cultivations. Cyanobacteria offer the advantage of being able to utilize both CO_2 and bicarbonate as sources of inorganic carbon. Intercellular bicarbonate is converted into CO_2 *via* the carbonic anhydrase system, creating a relatively high concentration of CO_2 in the vicinity of ribulose bis-phosphate carboxylase-oxygenase (RuBisCO), resulting in the carboxylation reaction dominating over the counterproductive oxygenation property of this enzyme [64]. Inorganic carbon is also one of the greatest costs associated with scale-up for Isp production, and the CO_2 concentrations in the atmosphere (400 p.p.m.) [65] are too low to support high rates of growth. Cost-effective means of CO_2 administration would be coupling cultivation of the cyanobacteria with waste resources such as flue gases from power plants [66], biogas from anaerobic digestion of agricultural waste [67], or biogas from aerobic composting of animal manure [68]. The process of Isp production could thus work dually, as a means of bioremediation, preventing waste CO_2 from entering the atmosphere, and supporting high rates of cyanobacterial photosynthesis and growth.

Other macronutrients required for cyanobacterial growth include nitrogen and phosphorous. Nitrogen is an essential constitutent of many cellular compounds, comprising up to 10% of the cellular biomass [69–71]. Diazotrophic species of cyanobacteria offer the advantage of cultivation in nitrogen free media [72], potentially conserving resources and minimizing costs. Phosphorous makes up between 0.5% and 3.5% of the biomass, and is essential for the processes of photosynthesis and carbohydrate metabolism, in addition to the formation of nucleic acids, membrane phospholipids, and ATP [73]. Dissolved phosphates and phosphate bioavailability [58,74,75] are potential cultivation issues. Other macronutrients that are essential for cyanobacterial growth are: calcium, magnesium, potassium, and sulfur. Micronutrients required include boron, chloride, copper, cobalt, iron, manganese, molybdenum, nickel, and zinc [76].

The efficiency of cellular photosynthesis and the solar-to-biomass energy conversion efficiency of the culture are important productivity determinants. Cyanobacteria have developed an ancillary light-harvesting antenna system, the phycobilisome, comprising allophycocyanin core cylinders and their peripherally associated phycocyanin containing rods [77–81]. These large arrays of light-harvesting antenna complexes confer a survival advantage to the cells in the wild, when cyanobacteria grow in turbid waters, and where light intensity is low and limited for photosynthesis. However, under scale-up conditions, cultures would be grown under direct sunlight in regions with high intensities of illumination. Under these conditions, the rate of light absorption far exceeds the capacity of cellular photosynthesis to utilize incoming photons, and the majority (80%) of absorbed photons would be lost through nonphotochemical quenching [82–84]. Engineering of the photosynthetic antenna for high density mass cultures has shown to improve biomass yields in green microalgae [85], cyanobacteria [86,87], and a crop plant [88]. This approach involves a genetic truncation of the light-harvesting antenna to prevent excess sunlight absorption by individual photosystems and also to minimize shading among the cells in the medium, thereby increasing the penetration of sunlight through the high density culture to reach more cells per volume in the culture [85,86]. In cyanobacteria, this was achieved by deleting the *cpc* operon that encodes for the multiple phycocyanin rods, resulting in a Δ*cpc* mutant lacking phycocyanin and achieving up to 57% greater rates in biomass accumulation and yield under bright growth irradiance. Deletion of phycocyanin may also have a positive impact on product generation, as it alleviates investment of endogenous substrate in protein and pigment associated with the peripheral light-harvesting antenna. In an Isp-producing strain of *Synechocystis*, removal of the light-harvesting phycocyanin rods increased Isp production (Isp : biomass; mg : g) by up to fivefold, potentially due to an increase in cellular endogenous metabolites as a result of the lack of phycocyanin biosynthesis [89]. The design, structure, and materials of suitable photobioreactors will also dictate how sunlight is

absorbed and utilized by the culture, and can be devised around the purpose of optimizing light absorption for photosynthetic efficiency [39,89–94].

Overcoming limitations in substrate flux & enzyme concentration

Isoprene biosynthesis is dependent upon the capacity of the cyanobacteria to generate DMAPP through the native MEP pathway, and/or through a heterologous MVA pathway. Recently, a metabolite quantitation of the precursors required for both the mevalonate (MVA), and the MEP pathways have been conducted in *S. elongatus* PCC 7942 grown under continuous illumination [30]. It was found that the intracellular pool size of the precursors for the MEP pathway, G3P and pyruvate were 51.3 ± 5.1 μM, and 1.85.3 ± 217.8 mM respectively. The intracellular concentration of acetyl-CoA for the MVA pathway was 53.6 ± 1.7 μM [30]. For each molecule of DMAPP, one G3P and one pyruvate molecule are required in the MEP pathway, versus three molecules of acetyl-CoA required in the MVA pathway. Therefore, it is advantageous for the cyanobacteria to employ the MEP pathway as the main driver of Isp production during photoautotrophic growth. However, a heterologous MVA pathway has been engineered to work in concert with the MEP pathway in *Synechocystis* sp PCC 6803, enhancing the rate and yield of Isp production [29]. This was the first report expressing an entire biosynthetic pathway consisting of the seven MVA genes into a photosynthetic microorganism, and generated improvements in Isp production by up to 2.5-fold [29].

It has been established in heterotrophic microorganisms that the MEP pathway is limited by the activity of the first enzyme, Dxs, in the process (Fig. 1) [95,96]. In cyanobacteria, it has been shown that the Ipi encoded by the *Ipi* gene is also limiting Isp production. Intracellular concentration ratios of DMAPP/IPP in *Synechococcus* sp. PCC 7942 were measured to be 1 : 2.7. Experimental overexpression of the Ipi resulted in enhanced Isp production by a 2–3 fold in *Synechocystis* [89] and *Synechococcus* [30], indicating that the intracellular DMAPP levels are limiting the IspS reaction. Fusing the IspS directly to the Ipi also increased Isp production, likely due to direct channelling of DMAPP from the Ipi, into the IspS [30]. Kinetic flux analysis of the endogenous MEP pathway in *Synechococcus* sp. PCC 7942 was also conducted to determine additional bottleneck enzymes in a photosynthetic cell, and found the step encoded by the *IspG* gene (Fig. 1) to be rate limiting. Overexpression of the *IspG* increased Isp production by up to 60% [30]. The creation of a strain combining the expression of an Ipi-IspS fusion + DXS + IspG resulted in significant Isp production rates of 0.54 $g{\cdot}g^{-1}$ (Isp : biomass; w : w) [30]. However, validation of expression of the Dxs and IspG genes by protein analysis were not shown, and the DNA sequences of the heterologous genes incorporated in *Synechococcus* sp. PCC 7942 were not reported in these studies.

The plant IspSs, like all the terpene synthases, is notoriously slow enzymes that have been identified and characterized in multiple species [97–103]. Due to their very slow K_{cat}, relatively high K_m, and poor expression levels in cyanobacteria, the IspS itself is a limiting factor toward photosynthetic Isp production. A number of known IspSs have been expressed and tested in *Synechococcus* sp. PCC 7942 including: *P. montana, Populus trichocarpa, Populus deltoides, Populus alba, Populus canescens, and Eucalyptus globulus* [30]. The *P. montana* IspS is the only expressed and systematically tested in *Synechocystis* sp. PCC 6803 so far [19,29,39,40,62,89,104]. The *Synechococcus* sp. PCC 7942 codon-optimized *E. globulus* IspS was reported to have the highest catalytic activity as of yet, generating 107 $\mu g{\cdot}Isp{\cdot}h^{-1}{\cdot}L^{-1}$ culture under *in vivo* conditions [30]. The codon-optimized nucleotide or amino acid sequence of this *IspS* has not been published or verified beyond this single study so far. Protein engineering toward increased *IspS* catalysis has also been of interest in the field. Recently, directed evolution of an IspS using a DMAPP toxicity screen successfully improved rates of Isp production in *Saccharomyces cerevisiae* by up to 1.8-fold [105].

Fusion constructs as protein overexpression vectors

To counter the slow catalytic activity of the IspS enzyme, efforts have been made to increase its cellular concentration. In this endeavor, fusion constructs of the *IspS* gene to the cyanobacterial *cpcB* gene, encoding the abundant β-subunit of phycocyanin, proved to substantially overexpress the IspS protein by up to 275-fold, making the cpcB*IspS fusion the most abundant protein in the cell [104]. This technique was previously shown to successfully overexpress other terpene synthases in *Synechocystis* [106,107]. The rationale behind the move to overexpress the IspS is that terpene synthases, including the IspS, have a slow K_{cat} (V_{max}) necessitating high levels of enzyme concentration to enable greater rates and yields of product formation. Fusion of the IspS to the β-subunit of phycocyanin caused a slower enzymatic specific activity, but still resulted in an overall increase in Isp

production due to the greater abundance of the enzyme. Yields increased from 0.2 $mg \cdot g^{-1}$ Isp : biomass (w : w) in the unfused control, to ~ 5.4 $mg \cdot g^{-1}$·Isp : biomass (w : w) in the cpcB*IspS fusion transformant, a 27-fold greater yield, without entailing application of any other metabolic improvements [104]. This cpcB*IspS fusion construct had a specific activity of only about 10% of that measured with the free enzyme, so there is potential for increased productivity by up to 10-fold upon further engineering of the cpcB*IspS fusion linkage to alleviate steric hindrances and to improve the natural activity of the fusion construct toward K_{cat} values that were measured with the native form of the IspS enzyme [97].

Conclusion

Cyanobacterial production of Isp is a promising lead technology for the future of renewable chemicals. Further improvements in rates and yield promise to make bio-based Isp competitive with that derived from fossil sources. Cyanobacteria-based Isp production has yet to be reported at pilot or large scale. The migration of current proof-of-concept lab protocols into working commercial production systems remains to be tested and validated with large volume enclosed (tubular) photo-bioreactors suitable for this technology. This would require the endowment of the IspS properties to a competent commercial strain having properties of high temperature and irradiance tolerance, and one that can grow in saline, alkaline, and wastewater, in a cost-effective photo-bioreactor.

Future improvements in the metabolic generation of Isp may include a synthetic biology approach using combinatorial optimizations of heterologous pathways, and down regulation of competing pathways, in concert with global analysis of the impact on cellular metabolites [108,109]. The ultimate objective in this endeavor is to convert the slow secondary metabolism of the terpenoid biosynthetic pathway into the dominant metabolic activity of the cyanobacterial cell, essentially directing most of the photosynthetically reduced CO_2 toward Isp or other useful isoprenoid products.

Acknowledgements

The work was conducted in partial satisfaction of the requirements for the degree of Doctor of Philosophy by JEC, supported by a graduate student fellowship from the NSF Sage IGERT program.

Author contributions

JEC wrote this Review and AM edited the manuscript.

References

1 Pimentel D, Marklein A, Toth MA, Karpoff MN, Paul GS, McCormack R, Kyriazis J and Krueger T (2009) Food versus biofuels: environmental and economic costs. *Hum Ecol* **37**, 1.

2 Stephens E, Ross IL, Mussgnug JH, Wagner LD, Borowitzka MA, Posten C, Kruse O and Hankamer B (2010) Future prospects of microalgal biofuel production systems. *Trends Plant Sci* **15**, 554–564.

3 Morais ARC, Dworakowska S, Reis A, Gouveia L, Matos CT, Bogdał D and Bogel-Łukasik R (2015) Chemical and biological-based isoprene production: green metrics. *Catal Today* **239**, 38–43.

4 Logan BA, Monson RK and Potosnak MJ (2000) Biochemistry and physiology of foliar isoprene production. *Trends Plant Sci* **5**, 477–481.

5 Sharkey TD and Loreto F (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. *Oecologia* **95**, 328–333.

6 Harley PC, Monson RK and Lerdau MT (1999) Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia* **118**, 109–123.

7 Loreto F and Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol* **127**, 1781–1787.

8 Guenther A, Karl T, Harley P, Wiedinmyer C, Palmer PI and Geron C (2006) Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature) Estimates of global terrestrial iso- prene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmos Chem Phys* **6**, 3181–3210.

9 Lichtenthaler HK (2007) Biosynthesis, accumulation and emission of carotenoids, α-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. *Photosynth Res* **92**, 163–179.

10 Lichtenthaler HK (2010) The non-mevalonate DOXP/MEP (deoxyxylulose 5-phosphate/methylerythritol 4-phosphate) pathway of chloroplast isoprenoid and pigment biosynthesis. The Chloroplast, pp. 95–118. Springer, Dordrecht.

11 Steinbü A (2003) Production of rubber-like polymers by microorganisms. *Curr Opin Microbiol* **6**, 261–270.

12 Greve H-H, ed. (2000) Rubber, 2. Natural. In Ullmann's Encyclopedia of Industrial Chemistry, pp. 583–594. Wiley-VCH Verlag GmbH & Co. KGaA., Weinheim.
13 Tupy J (1973) The regulation of invertase activity in the latex of *Hevea brasiliensis* Muell. *Argic J Exp Bot* **24**, 516–524.
14 Senevirathna AMWK, Stirling CM and Rodrigo VHL (2003) Growth, photosynthetic performance and shade adaptation of rubber (Hevea brasiliensis) grown in natural shade. *Tree Physiol* **23**, 705–712.
15 Knoot CJ, Ungerer JL, Wangikar PP and Pakrasi HB (2018) Cyanobacteria: promising biocatalysts for sustainable chemical production. *J Biol Chem* **293**, 5044–5052.
16 Duchoud F, Chuang DSW and Liao JC (2016) Cyanobacteria as a host organism. Industrial Biotechnology, pp. 581–604. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
17 Zhu X-G, Long SP and Ort DR (2008) What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Curr Opin Biotechnol* **19**, 153–159.
18 Melis A (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. *Plant Sci* **177**, 272–280.
19 Lindberg P, Park S and Melis A (2010) Engineering a platform for photosynthetic isoprene production in cyanobacteria, using *Synechocystis* as the model organism. *Metab Eng* **12**, 70–79.
20 Pemberton TA, Chen M, Harris GG, Chou WKW, Duan L, Köksal M, Genshaft AS, Cane DE and Christianson DW (2017) Exploring the influence of domain architecture on the catalytic function of diterpene synthases. *Biochemistry* **56**, 2010–2023.
21 McGarvey DJ and Croteau R (1995) Terpenoid metabolism. *Plant Cell* **7**, 1015–1026.
22 McCaskill D and Croteau R (1997) Prospects for the bioengineering of isoprenoid biosynthesis. Advances in Biochemical Engineering/Biotechnology, pp. 107–146. Springer, Berlin, Heidelberg.
23 Wong J, Rios-Solis L and Keasling JD (2017) Microbial production of isoprenoids. In Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Production of Fuels and Chemicals (Lee SY, ed). pp. 1–24. Springer International Publishing, Chim.
24 Herrmann A (2011) The Chemistry and Biology of Volatiles. John Wiley & Sons, Inc., Chichester, UK.
25 Tholl D (2015) Biosynthesis and biological functions of terpenoids in plants. In Biotechnology of Isoprenoids (Schrader J and Bohlmann J, eds). pp. 63–106. Springer, Cham.
26 Arigoni D, Sagner S, Latzel C, Eisenreich W, Bacher A and Zenk MH (1997) Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. *Proc Natl Acad Sci USA* **94**, 10600–10605.
27 Eisenreich W, Schwarz M, Cartayrade A, Arigoni D, Zenk MH and Bacher A (1998) The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chem Biol* **5**, R221–R233.
28 Zhao Y, Yang J, Qin B, Li Y, Sun Y, Su S and Xian M (2011) Biosynthesis of isoprene in *Escherichia coli* via methylerythritol phosphate (MEP) pathway. *Appl Microbiol Biotechnol* **90**, 1915–1922.
29 Bentley FK, Zurbriggen A and Melis A (2014) Heterologous expression of the mevalonic acid pathway in cyanobacteria enhances endogenous carbon partitioning to isoprene. *Mol Plant* **7**, 71–86.
30 Gao X, Gao F, Liu D, Zhang H, Nie X and Yang C (2016) Engineering the methylerythritol phosphate pathway in cyanobacteria for photosynthetic isoprene production from CO_2. *Energy Environ Sci* **9**, 1400–1411.
31 Steinhauser D, Fernie AR and Araújo WL (2012) Unusual cyanobacterial TCA cycles: not broken just different. *Trends Plant Sci* **17**, 503–509.
32 Zhang S and Bryant DA (2011) The tricarboxylic acid cycle in cyanobacteria. *Science* **334**, 1551–1553.
33 Anfelt J, Kaczmarzyk D, Shabestary K, Renberg B, Rockberg J, Nielsen J, Uhlén M and Hudson EP (2015) Genetic and nutrient modulation of acetyl-CoA levels in *Synechocystis* for n-butanol production. *Microb Cell Fact* **14**, 167.
34 Barkley SJ, Desai SB and Poulter CD (2004) Type II isopentenyl diphosphate isomerase from *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **186**, 8156–8158.
35 Melis A (2013) Carbon partitioning in photosynthesis. *Curr Opin Chem Biol* **17**, 453–456.
36 Oliver JWK and Atsumi S (2014) Metabolic design for cyanobacterial chemical synthesis. *Photosynth Res* **120**, 249–261.
37 Shikanai T, Munekage Y, Kimura K, Shikanai T, Munekage Y and Kimura K (2002) Regulation of proton-to-electron stoichiometry in photosynthetic electron transport: physiological function in photoprotection. *J Plant Res* **115**, 3–10.
38 Vermaas W (2013) Solar-powered production of biofuels and other petroleum substitutes by cyanobacteria: stoichiometries of reducing equivalents and chemical energy, and energy conversion efficiency. Photosynthesis Research for Food, Fuel and the Future, pp. 353–357. Springer, Berlin, Heidelberg.
39 Bentley FK and Melis A (2012) Diffusion-based process for carbon dioxide uptake and isoprene emission in gaseous/aqueous two-phase photobioreactors by photosynthetic microorganisms. *Biotechnol Bioeng* **109**, 100–109.

40 Pade N, Erdmann S, Enke H, Dethloff F, Dühring U, Georg J, Wambutt J, Kopka J, Hess WR, Zimmermann R *et al*. (2016) Insights into isoprene production using the cyanobacterium *Synechocystis* sp. PCC 6803. *Biotechnol Biofuels* **9**, 89.

41 Molina Grima E, Belarbi E-H, Acién Fernández F, Robles Medina A and Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* **20**, 491–515.

42 Dittmann E, Gugger M, Sivonen K and Fewer DP (2015) Natural product biosynthetic diversity and comparative genomics of the cyanobacteria. *Trends Microbiol* **23**, 642–652.

43 Tan LT (2007) Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochemistry* **68**, 954–979.

44 Wagner J, Bransgrove R, Beacham TA, Allen MJ, Meixner K, Drosg B, Ting VP and Chuck CJ (2016) Co-production of bio-oil and propylene through the hydrothermal liquefaction of polyhydroxybutyrate producing cyanobacteria. *Bioresour Technol* **207**, 166–174.

45 Huang Y, Chen Y, Xie J, Liu H, Yin X and Wu C (2016) Bio-oil production from hydrothermal liquefaction of high-protein high-ash microalgae including wild cyanobacteria sp. and cultivated *Bacillariophyta* sp. *Fuel* **183**, 9–19.

46 Möllers K, Cannella D, Jørgensen H and Frigaard N-U (2014) Cyanobacterial biomass as carbohydrate and nutrient feedstock for bioethanol production by yeast fermentation. *Biotechnol Biofuels* **7**, 64.

47 Chow T-J, Su H-Y, Tsai T-Y, Chou H-H and Lee T-M (2015) Using recombinant cyanobacterium (*Synechococcus elongatus*) with increased carbohydrate productivity as feedstock for bioethanol production via separate hydrolysis and fermentation process. *Bioresour Technol* **184**, 33–41.

48 Tsygankov AA, Fedorov AS, Kosourov SN and Rao KK (2002) Hydrogen production by cyanobacteria in an automated outdoor photobioreactor under aerobic conditions. *Biotechnol Bioeng* **80**, 777–783.

49 Tredici MR, Carlozzi P, Chini Zittelli G and Materassi R (1991) A vertical alveolar panel (VAP) for outdoor mass cultivation of microalgae and cyanobacteria. *Bioresour Technol* **38**, 153–159.

50 Borodin VB, Tsygankov AA, Rao KK and Hall DO (2000) Hydrogen production by *Anabaena variabilis* PK84 under simulated outdoor conditions. *Biotechnol Bioeng* **69**, 478–485.

51 Deng Z, Yan C, Lu F, Hu Q and Hu Z (2008) Growth kinetics of 1–2 mm and 3–4 mm colonies of *Nostoc sphaeroides* (Cyanophyta) in outdoor culture. *Biotechnol Lett* **30**, 1741–1746.

52 Reichert CC, Reinehr CO and Costa JAV (2006) Semicontinuous cultivation of the cyanobacterium *Spirulina platensis* in a closed photobioreactor. *Braz J Chem Eng* **23**, 23–28.

53 Zhang L, Chen L, Wang J, Chen Y, Gao X, Zhang Z and Liu T (2015) Attached cultivation for improving the biomass productivity of *Spirulina platensis*. *Bioresour Technol* **181**, 136–142.

54 Thajuddin N and Subramanian G (2005) Cyanobacterial biodiversity and potential applications in biotechnology. *Curr Sci* **89**, 47–57.

55 Jordan A, Chandler J, MacCready JS, Huang J, Osteryoung KW and Ducat DC (2017) Engineering cyanobacterial cell morphology for enhanced recovery and processing of biomass. *Appl Environ Microbiol* **83**, e00053-17.

56 Smith VH and Crews T (2014) Applying ecological principles of crop cultivation in large-scale algal biomass production. *Algal Res* **4**, 23–34.

57 Newby DT, Mathews TJ, Pate RC, Huesemann MH, Lane TW, Wahlen BD, Mandal S, Engler RK, Feris KP and Shurin JB (2016) Assessing the potential of polyculture to accelerate algal biofuel production. *Algal Res* **19**, 264–277.

58 Markou G and Georgakakis D (2011) Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review. *Appl Energy* **88**, 3389–3401.

59 Seckbach J (2007) Algae and Cyanobacteria in Extreme Environments. Springer, Dordrecht, Netherlands.

60 Paerl HW, Pinckney JL and Steppe TF (2000) Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. *Environ Microbiol* **2**, 11–26.

61 Fritsen CH and Priscu JC (1998) Cyanobacterial assemblages in permanent ice covers on Antarctic lakes: distribution, growth rate, and temperature response of photosynthesis. *J Phycol* **34**, 587–597.

62 Chaves JE, Kirst H and Melis A (2015) Isoprene production in *Synechocystis* under alkaline and saline growth conditions. *J Appl Phycol* **27**, 1089–1097.

63 Su H-Y, Chou H-H, Chow T-J, Lee T-M, Huang W-L and Chen H-J (2017) Improvement of outdoor culture efficiency of cyanobacteria by over-expression of stress tolerance genes and its implication as bio-refinery feedstock. *Bioresour Technol* **244**, 1294–1303.

64 Kaplan A, Schwarz R, Lieman-Hurwitz J and Reinhold L (1991) Physiological and molecular aspects of the inorganic carbon-concentrating mechanism in cyanobacteria. *Plant Physiol* **97**, 851–855.

65 van Groenigen KJ, Osenberg CW, Terrer C, Carrillo Y, Dijkstra FA, Heath J, Nie M, Pendall E, Phillips RP and Hungate BA (2017) Faster turnover of new soil carbon inputs under increased atmospheric CO_2. *Glob Chang Biol* **23**, 4420–4429.

66 Vuppaladadiyam AK, Yao JG, Florin N, George A, Wang X, Labeeuw L, Jiang Y, Davis RW, Abbas A, Ralph P *et al.* (2018) Impact of flue gas compounds on microalgae and mechanisms for carbon assimilation and utilization. *Chemsuschem* **11**, 334–355.

67 Travieso L, Sanchez E, Benitez F and Conde JL (1993) *Arthospira* sp. intensive cultures for food and biogas purification. *Biotechnol Lett* **15**, 1091–1094.

68 Venkataraman LV, Madhavi Devi K, Mahadevaswamy M and Mohammed Kunhi AA (1982) Utilisation of rural wastes for algal biomass production with *Scenedesmus acutus* and *Spirulina platensis* in India. *Agric Wastes* **4**, 117–130.

69 Vieira Costa JA, Cozza KL, Oliveira L and Magagnin G (2001) Different nitrogen sources and growth responses of *Spirulina platensis* in microenvironments. *World J Microbiol Biotechnol* **17**, 439–442.

70 Benemann JR (1979) Production of nitrogen fertilizer with nitrogen-fixing blue-green algae. *Enzyme Microb Technol* **1**, 83–90.

71 Belkin S and Boussiba S (1991) Resistance of *Spirulina platensis* to ammonia at high pH values. *Plant Cell Physiol* **32**, 953–958.

72 Moreno J, Vargas MA, Rodríguez H, Rivas J and Guerrero MG (2003) Outdoor cultivation of a nitrogen-fixing marine cyanobacterium *Anabaena* sp. ATCC 33047. *Biomol Eng* **20**, 191–197.

73 Geider R and La Roche J (2002) Redfield revisited: variability of C:N: P in marine microalgae and its biochemical basis. *Eur J Phycol* **37**, 1–17.

74 Lodi A, Binaghi L, Solisio C, Converti A and Del Borghi M (2003) Nitrate and phosphate removal by *Spirulina platensis*. *J Ind Microbiol Biotechnol* **30**, 656–660.

75 Whitton BA and Potts M (2000) The Ecology of Cyanobacteria : Their Diversity in Time and Space. Kluwer Academic, New York, NY.

76 Eaton-Rye JJ (2004) The construction of gene knockouts in the cyanobacterium *Synechocystis* sp. PCC 6803. *Photosynth Res Protoc* **274**, 309–324.

77 Arteni AA, Ajlani G and Boekema EJ (2009) Structural organisation of phycobilisomes from *Synechocystis* sp. strain PCC6803 and their interaction with the membrane. *Biochim Biophys Acta* **1787**, 272–279.

78 Liu H, Zhang H, Niedzwiedzki DM, Prado M, He G, Gross ML and Blankenship RE (2013) Phycobilisomes supply excitations to both photosystems in a megacomplex in cyanobacteria. *Science* **342**, 1104–1107.

79 Glazer AN (1985) Light harvesting by phycobilisomes. *Annu Rev Biophys Biophys Chem* **14**, 47–77.

80 Gantt E (1981) Phycobilisomes. *Annu Rev Plant Physiol* **32**, 327–347.

81 Bryant DA, Guglielmi G, de Marsac NT, Castets A-M and Cohen-Bazire G (1979) The structure of cyanobacterial phycobilisomes: a model. *Arch Microbiol* **123**, 113–127.

82 Bailey S and Grossman A (2008) Photoprotection in cyanobacteria: regulation of light harvesting†. *Photochem Photobiol* **84**, 1410–1420.

83 Kirilovsky D and Kerfeld CA (2012) The orange carotenoid protein in photoprotection of photosystem II in cyanobacteria. *Biochim Biophys Acta* **1817**, 158–166.

84 Kirilovsky D (2007) Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism. *Photosynth Res* **93**, 7–16.

85 Kirst H and Melis A (2014) The chloroplast signal recognition particle (CpSRP) pathway as a tool to minimize chlorophyll antenna size and maximize photosynthetic productivity. *Biotechnol Adv* **32**, 66–72.

86 Kirst H, Formighieri C and Melis A (2014) Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. *Biochim Biophys Acta* **1837**, 1653–1664.

87 Kwon J-H, Bernát G, Wagner H, Rögner M and Rexroth S (2013) Reduced light-harvesting antenna: consequences on cyanobacterial metabolism and photosynthetic productivity. *Algal Res* **2**, 188–195.

88 Kirst H, Gabilly ST, Niyogi KK, Lemaux PG and Melis A (2017) Photosynthetic antenna engineering to improve crop yields. *Planta* **245**, 1009–1020.

89 Chaves JE, Romero PR, Kirst H and Melis A (2016) Role of isopentenyl-diphosphate isomerase in heterologous cyanobacterial (*Synechocystis*) isoprene production. *Photosynth Res* **130**, 517–527.

90 Watanabe Y, de la Noüe J and Hall DO (1995) Photosynthetic performance of a helical tubular photobioreactor incorporating the cyanobacterium *spirulina platensis*. *Biotechnol Bioeng* **47**, 261–269.

91 Posten C (2009) Design principles of photo-bioreactors for cultivation of microalgae. *Eng Life Sci* **9**, 165–177.

92 Carvalho AP, Meireles LA and Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol Prog* **22**, 1490–1506.

93 Suh IS and Lee C-G (2003) Photobioreactor engineering: design and performance. *Biotechnol Bioprocess Eng* **8**, 313–321.

94 Janssen M, Tramper J, Mur LR and Wijffels RH (2003) Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnol Bioeng* **81**, 193–210.

95 Lv X, Gu J, Wang F, Xie W, Liu M, Ye L and Yu H (2016) Combinatorial pathway optimization in *Escherichia coli* by directed co-evolution of rate-limiting enzymes and modular pathway engineering. *Biotechnol Bioeng* **113**, 2661–2669.

96 Matthews PD and Wurtzel ET (2000) Metabolic engineering of carotenoid accumulation in *Escherichia coli* by modulation of the isoprenoid precursor pool with expression of deoxyxylulose phosphate synthase. *Appl Microbiol Biotechnol* **53**, 396–400.
97 Zurbriggen A, Kirst H and Melis A (2012) Isoprene production via the mevalonic acid pathway in *Escherichia coli* (Bacteria). *Bioenergy Res* **5**, 814–828.
98 Silver GM and Fall R (1995) Characterization of aspen isoprene synthase, an enzyme responsible for leaf isoprene emission to the atmosphere. *J Biol Chem* **270**, 13010–13016.
99 Sasaki K, Ohara K and Yazaki K (2005) Gene expression and characterization of isoprene synthase from *Populus alba*. *FEBS Lett* **579**, 2514–2518.
100 Lehning A, Zimmer I, Steinbrecher R, Brüggemann N and Schnitzler J-P (1999) Isoprene synthase activity and its relation to isoprene emission in *Quercus robur* L. leaves. *Plant, Cell Environ* **22**, 495–504.
101 Silver GM, Fall R, Crowell D, Randall S, Lazaro J and Gorge J (1991) Enzymatic synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts. *Plant Physiol* **97**, 1588–1591.
102 Köksal M, Zimmer I, Schnitzler J-P and Christianson DW (2010) Structure of isoprene synthase illuminates the chemical mechanism of teragram atmospheric carbon emission. *J Mol Biol* **402**, 363–373.
103 Sharkey TD, Gray DW, Pell HK, Breneman SR and Topper L (2013) Isoprene synthase genes form a monophyletic clade of acyclic terpene synthases in the TPS-B terpene synthase family. *Evolution (N. Y)* **67**, 1026–1040.
104 Chaves JE, Rueda-Romero P, Kirst H and Melis A (2017) Engineering isoprene synthase expression and activity in cyanobacteria. *ACS Synth Biol* **6**, 2281–2292.
105 Wang F, Lv X, Xie W, Zhou P, Zhu Y, Yao Z, Yang C, Yang X, Ye L and Yu H (2017) Combining Gal4p-mediated expression enhancement and directed evolution of isoprene synthase to improve isoprene production in *Saccharomyces cerevisiae*. *Metab Eng* **39**, 257–266.
106 Formighieri C and Melis A (2015) A phycocyanin·phellandrene synthase fusion enhances recombinant protein expression and β-phellandrene (monoterpene) hydrocarbons production in *Synechocystis* (cyanobacteria). *Metab Eng* **32**, 116–124.
107 Formighieri C and Melis A (2017) Heterologous synthesis of geranyllinalool, a diterpenol plant product, in the cyanobacterium *Synechocystis*. *Appl Microbiol Biotechnol* **101**, 2791–2800.
108 Stephanopoulos G (2012) Synthetic biology and metabolic engineering. *ACS Synth Biol* **1**, 514–525.
109 Moses T, Mehrshahi P, Smith AG and Goossens A (2017) Synthetic biology approaches for the production of plant metabolites in unicellular organisms. *J Exp Bot* **68**, 4057–4074.