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Engineering biology approaches for food and nutrient production by cyanobacteria

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As photoautotrophic organisms, cyanobacteria capture and store solar energy in the form of biomass. Cyanobacterial biomass has been an important component of diet and nutrition in several regions for centuries. Synthetic biology strategies are currently being applied to increase the yield and productivity of cyanobacterial biomass by optimizing solar energy utilization and CO_2 fixation rates for carbon storage. Likewise, engineering cyanobacteria as cellular factories to synthesize carbohydrates, amino acids, proteins, lipids and fatty acids is providing an attractive way to sustainably produce food and nutrients for human consumption. In this review, we have summarized recent progress in both aspects and prospective trends under development.

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Introduction

Cyanobacteria are photoautotrophic microbes that have played a major role in shaping the Earth's biosphere. Cyanobacteria were responsible for the increase in oxygen in the atmosphere more than 2 billion years ago, known as the great oxygenation event, which facilitated the proliferation of eukaryotes [1]. The ability of cyanobacteria to convert carbon dioxide and sunlight into oxygen and bio-products such as nutrients and biofuels makes them critical for numerous biotechnological purposes [2]. Cyanobacteria are increasingly being used for production of food and nutrition in important industrial settings. In the face of human population growth and concomitant pressure on natural resource utilization, cyanobacteria are promising candidates for expanded applications due to their ability to grow without using arable lands and potential to use industrial waste products [3].

Cyanobacteria are prokaryotic organisms that use phycobilisomes, large pigment-protein antenna complexes, to harvest light energy and transfer it to reaction centers where photochemical reactions occur. Cyanobacteria contain a large internal thylakoid membrane system where the components of the photosynthetic electron transfer system are located, including photosystems I and II (PSI and PSII) [4]. These bacterial cells also contain numerous inclusion bodies and compartments, including the carboxysome, which facilitates the carbon-concentrating mechanism (CCM) that is important for efficient carbon fixation [5]. Cyanobacteria are found in a wide range of habitats, from open ocean to desert crusts. Their metabolic versatility lends these organisms to bioengineering applications for sustainable production of a wide variety of products. This review discusses important recent advances in engineering to improve cyanobacterial biomass production and manipulate cyanobacteria as cellular factories to produce food and nutrients.

Cyanobacterial biomass used as food

There are multiple records of historical usage of cyanobacteria and microalgae in human diets. *Arthrospira* (commercially known as *Spirulina*) is a well-known cyanobacterium that was consumed long ago around Chad Lake in Africa and by the Aztecs in Central Mexico [6]. Other filamentous cyanobacteria that are widely used as food in Asia and South America belong to the genus *Nostoc* [7,8]. At present, more than 70 countries have commercialized products of nutritional importance that are obtained from cyanobacteria [9]. Biomass of cyanobacteria contains high amounts of protein and other nutritional components. For instance, *Arthrospira* reaches 50–70% protein and produces fatty polyunsaturated acids (1.5–2%), lipids (5–6%) as well as various vitamins [6]. Several studies and reviews have summarized the strategies of cultivation and process optimization to improve the production of cyanobacterial biomass [10–13]. Here, we are focusing on studies using synthetic biology (mainly through genetic engineering) to enhance biomass yield and biomass productivity, which is determined by photosynthesis and growth rate, respectively.

Engineering to increase biomass yield

Microbial bioengineering is dominated by organisms that are easy to genetically manipulate and display fast growth rates, such as *Escherichia coli* and yeast. In comparison, cyanobacteria are less well understood [14]. However, recent progress has shown that cyanobacteria are capable of fast growth and many strains are amenable to genetic modification [14]. CRISPR strategies have been applied in several strains of cyanobacteria to implement genetic changes [15].

To increase cyanobacterial biomass yield, efficient absorption of solar energy and fixation of CO_2 are two targets for engineering. Recent efforts to increase photosynthetic efficiency have focused on expansion of portion of the solar spectrum harvested and increasing electron transport chain activity. To absorb far-red light of wavelengths over 700 nm, chlorophyll *f* (Chl *f*) encoding genes were successfully introduced into the model species *Synechococcus* sp. PCC 7002 [16•]. After optimization of light growth conditions, Chl *f* was functionally integrated into PSI complexes and connected to the reaction center, resulting the extension of the active radiation for the new hybrid PSI complex up to 750 nm [17]. This expansion of the usable area of the solar spectrum has provided an advantage under non-saturating light conditions. Hasunuma *et al.* overexpressed the gene *flv3*, encoding an NAD(P)H:oxygen oxidoreductase in *Synechocystis* sp. PCC 6803, resulting in improved biomass accumulation by 30% in a week of culturing [18]. The study revealed that Flv3 overexpression improved the electron transport chain activity and ATP supply through the regeneration of $NADP^+$. Genes involved in the Calvin cycle and CCM are targets for engineering to improve the carbon fixation rate. Overexpression of RuBisCo, sedoheptulose 1,7-biphosphatase, transketolase and fructose-bisphosphate aldolase in both *Synechocystis* 6803 and *Synechococcus* 7002 proved to be effective to improve biomass accumulation [19,20]. Meanwhile, overexpression of the carbon transporters BicA and SbtA involved in CCM improved the biomass yield by 50–100% [21,22]. The progress to deeply understand the mechanisms of photosynthesis and carbon fixation is also helpful for synthetic biology applications to improve the biomass yield of cyanobacteria.

Exploring the fast growth in cyanobacteria

Although cyanobacteria offer attractive systems for biotechnological applications due to their increased growth rate compared to plants, the growth rates of commonly used cyanobacterial model strains are significantly slower compared to *E. coli* or yeast. For instance, *Synechocystis* 6803, *Synechococcus elongatus* PCC 7942 and *Synechococcus* 7002 are three model cyanobacterial strains used for decades, with doubling times under optimal conditions of 6.6 hours, 4.9 hours, and 4.1 hours, respectively [23]. Several studies have sought to identify and characterize new cyanobacterial strains that can grow rapidly as a superior phototrophic chassis for synthetic biology applications [23–26].

Isolated in India, *S. elongatus* PCC 11801 has a growth rate of 0.29 $hour^{-1}$ at 41°C and 1000 μmol photons m^{-2} s^{-1}, which is the highest reported for any cyanobacterium under ambient CO_2 conditions [24]. The same research group isolated and characterized another strain, *S. elongatus* PCC 11802, that is phylogenetically close to *S. elongatus* PCC 11801 with 97% genome identity [25]. The strain shows a doubling time of 2.8 hour under the optimal growth conditions of 1% CO_2, 38°C, and 1000 μmol photons m^{-2} s^{-1}. Isolated from Singapore, *Synechococcus* sp. PCC 11901 accumulates 1.7–3 times more biomass under optimized conditions than these commonly used model cyanobacteria. Its average biomass productivity reached 0.1 g CDW L^{-1} $hour^{-1}$ and the shortest doubling time is close to 2 hours at 30°C with 5% CO_2 [26].

With a 99.8% identical sequence to *S. elongatus* PCC 7942 (hereafter PCC 7942), *S. elongatus* UTEX 2973 (hereafter UTEX 2973) gained a large interest because of its much shorter doubling time [23]. At 42°C with 5% CO_2 and 1500 μmol photons m^{-2} s^{-1}, UTEX 2973 achieves the remarkable doubling time of 1.5 hour, which is comparable to the yeast strain *Saccharomyces cerevisiae* [27•]. There are only 55 single nucleotide differences between UTEX 2973 and PCC 7942 at the genome level, which provides an excellent opportunity to explore how a slower-growing organism can be transitioned into a faster-growing strain. Compared to PCC 7942, UTEX 2973 exhibits a 2.5-fold higher CO_2 uptake rate and a 1.9-fold-higher rate of O_2 evolution under culture conditions yielding the highest growth rates for each, indicating higher photosynthetic rates. These phenotypes in UTEX 2973 are tightly correlated to the increased rate of electron transfer from PSII and the content of PSI, cytochrome b_6f, and plastocyanin [27•]. Furthermore, to determine the specific loci out of 55 single nucleotide differences that confer rapid growth, a comprehensive mutational analysis of UTEX 2973 was preformed [28••]. Finally, three genes were identified, *atpA*, *ppnK* and *rpaA*, which encode the alpha subunit of ATP synthase, the NAD^+ kinase, and the global regulator of circadian clock output, respectively. Importantly, when the genome sequence of PCC 7942 at five loci in these 3 genes was converted to the UTEX 2973 sequence, the doubling time decreased to 2.3 hours. Commensurate with the increase in growth rate, the photosynthetic rate was also doubled in the fast growing PCC 7942 mutant [28••]. This study revealed the molecular basis for rapid growth and suggested that increased production of intracellular ATP and NADPH is directly responsible for rapid autotrophic growth.

Along with new species isolated recently, efforts have also been made to improve the growth rate of model species of

cyanobacteria. Van Alphen *et al.* identified sulfate and iron as limiting factors in the medium for *Synechocystis* 6803. After optimizing the growth conditions, the doubling time of *Synechocystis* 6803 was significantly shorten to 4.3 hours [29]. Bernstein *et al.* identified key mechanisms that allow *Synechococcus* 7002 to achieve fast growth with a 2.5 hour doubling time [30]. Such enhanced growth was supported by high rates of photosynthetic electron transfer, which corresponds well with the findings in UTEX 2973. Furthermore, Bernstein *et al.* found that significantly elevated transcription of precursor biosynthesis and protein translation machinery also contributed to the ultrafast growth of *Synechococcus* 7002 [30].

Computational model-based insights on the fast growth of cyanobacteria

Mueller *et al.* used genome scale metabolic models of UTEX 2973 and PCC 7942 to identify the factors that contributed to the fast growth phenotype [31]. Their analysis revealed that the major factor is the difference in their carbon uptake rates, which was supported by a later study based on genome scale ^{13}C-MFA of UTEX 2973 [32], and is consistent with the results observed in the study mentioned above [27•]. Reimers *et al.* used a genome scale dynamic resource allocation model to understand the optimal allocation of cellular resources during diurnal growth of PCC 7942 [33•]. In their analysis, the major determinant of growth rate was found to be the fraction of total proteome allocated for non-catalytic roles. This parameter determines the amount of cellular resources allocated for the synthesis of non-metabolic proteins that do not perform any catalytic function but still constitute the active proteome of the cell. For slower growing cells of PCC 7942 this fraction was calculated to be 0.55 [33•]. The lower this fraction, the higher the growth rate predicted by the model. Similar observations were also made in earlier modeling studies on cyanobacteria [34]. Detailed investigations of the above-mentioned model-based hypotheses would help to glean insights on the principles that govern growth rate, aiding in the engineering of strains with faster growth rates.

Production of nutrients from CO_2 using cyanobacterial cellular factories

Besides energy captured in the form of biomass as a food source, cyanobacteria have been used as green cellular factories to produce various nutrients, including carbohydrates, protein and amino acids, lipid and fatty acids, and pigments. Carbohydrates represent a major product of photosynthesis, with the content reaching up to 50% of dry weight in certain cyanobacteria [35]. Exopolysaccharide (EPS) has also been reported to contribute 25% of the total biomass of cyanobacteria [13]. Because of potential applications in the food industry, large-scale production of cyanobacterial EPS has received increasing attention. Most of cyanobacterial EPS is composed of six or more types of building blocks, of which glucose is frequently observed at the highest amount [36]. However, there is still poor knowledge of the structure of EPS and its production by cyanobacteria is a complex process that is regulated by many genes. Therefore, intensive studies are required for development of genetically engineered cyanobacteria for EPS production. Another carbohydrate that has gained interest is sucrose, which is naturally synthesized by many cyanobacteria under stress conditions and has been engineered for production in several species. In cyanobacteria, sucrose is synthesized from UDP-glucose and fructose-6-phosphate by sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP). Manipulation the expression of genes coding for these two enzymes has improved the accumulation of intracellular sucrose in several species, including *Synechocystis* 6803, PCC 7942, and UTEX 2973 [37–39]. Furthermore, introducing a sucrose permease (CscB) from *E. coli* into cyanobacteria has resulted in efficient export of sucrose [39]. To date, the highest production titer and productivity were reached in UTEX 2973 under salt stress conditions. After 5 days with 150 mM NaCl, 8 g L^{-1} sucrose was observed in the medium. The highest productivity during the process reached 1.9 g L^{-1} day^{-1}, which resulted in a sucrose yield of 3.1 g g^{-1} biomass [39].

A few studies have been done to produce specific proteins and amino acids using cyanobacteria as hosts. Phycobiliproteins have been widely used as a natural protein dye in the food industry, due to their water-soluble nature. The commercial production of phycobiliproteins is mainly restricted to *Arthrospira* (*Spirulina*), but Kumar Saini *et al.* explored its production in the diazotrophic cyanobacterium *Anabaena variabilis* CCC421 [40]. The group optimized the medium components resulting in enhancement of phycobiliprotein production from 190 mg/L to 408.5 mg/L, which demonstrated the potential to massively produce a specific protein in cyanobacteria. Cyanobacteria have also been engineered to produce several amino acids, including lysine in *Synechococcus* 7002 and aromatic amino acids (phenylalanine, tyrosine, and tryptophan) in *Synechocystis* 6803. Through the introduction of a lysine transporter YbjE and a feedback-inhibition-resistant aspartate kinase, and after the optimization of cultural conditions, engineered *Synechococcus* 7002 directed 18% of fixed carbon to lysine production, resulting a productivity of 72 mg L^{-1} day^{-1} [41]. Brey *et al.* engineered the metabolism of *Synechocystis* 6803 by heterologous expression of AroG and TyrA to produce phenylalanine and tyrosine, of which the yield reached 0.9 g g^{-1} cell dry weight and 0.064 g g^{-1} cell dry weight, respectively [42]. Combining random mutagenesis and AroG and TrpE overexpression, Deshpande *et al.* manipulated *Synechocystis* 6803 to produce tryptophan with a titer of 0.2 g L^{-1} after 10 days of autotrophic growth [43].

Many studies have been done in several species of cyanobacteria for lipid and fatty acid production, which

are recently systematically summarized in a review paper [44••]. Of particular interest is the production of the dietary omega-3 (n-3)polyunsaturated acids (PUFAs). To benefit human nutrition, health organizations have recommended increasing dietary consumption of n-3 PUFAs, leading to fast-growing markets in dietary supplements, functional foods, and infant formulas, especially for the long chain n-3 PUFAs eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) [45]. PUFAs are naturally produced by cyanobacteria as essential components and model species of cyanobacteria have been engineered to produce α-linolenic acid (ALA, 18:3), stearidonic acid (SDA, 18:4), and eicosatetraenoic acid (ETA, 20:4) [45–47]. The rate-limiting steps in intracellular biological conversion are the desaturation steps, and particularly the Δ6 desaturase. To date, the production yield of n-3 PUFAs in cyanobacteria is around 10−100 $mg\,g^{-1}$ cell dry weight. Poole *et al.* summarized all of the work related to PUFA production in cyanobacteria in a recent paper [45].

Carotenoids are light harvesting terpenoid pigments that have been studied as functional food supplements for decades due to potential health benefits [48]. The carotenoids produced by cyanobacteria include β-carotene, astaxanthin, zeaxanthin, and others [48]. β-carotene is used as a pro-vitamin A supplement and astaxanthin and zeaxanthin have commercial value in nutraceuticals due to antioxidant properties. Two decades ago, Lagarde *et al.* explored the potential to modify the content of carotenoids in *Synechocystis* 6803 [49]. Overexpression of IDI, CrtR, CrtP, and CrtB in the carotenoid biosynthetic pathway increased zeaxanthin accumulation by 2.5-fold and carotenoid content by 50%. Recently, astaxanthin was produced in both *Synechocystis* 6803 and *Synechococcus* 7002 [50,51•,52]. The highest yield of astaxanthin was 29.6 $mg\,g^{-1}$ cell dry weight reported in *Synechocystis* 6803. Diao *et al.* constructed an efficient astaxanthin anabolic pathway through multiple engineering steps to rewire endogenous metabolism, including introducing two key enzymes, β-carotenoid ketolase and hydroxylase, as well as screening and carbon flux enhancements for precursor supply in the native MEP pathway [51•].

Model-based tools for engineering

Synthetic biology efforts to overproduce nutrients from cyanobacteria can be guided by metabolic models. Both constraint-based metabolic models and kinetic models are already in use to guide genetic modifications for overproducing target chemicals in cyanobacteria [53]. High quality genome scale metabolic models are available for a number of model and non-model cyanobacteria [54], including *Arthrospira* [55]. For model organisms with well-developed genetic tools, recent GSM efforts had incorporated extensive experimental validation into their workflow [56]. Some of these models were used to improve the production alongside strain designing algorithms such as minimization of metabolic adjustments (MOMA) [57] and OptForce [58]. Kinetic modeling efforts on the other hand are confined only to the model strains *Synechocystis* 6803 and PCC 7942, although state-of-the-art parameterization techniques allow for the development of such models in other less studied cyanobacteria [53]. In cyanobacteria, this modeling framework has proven useful in enhancing the production of target chemicals [59,60]. Another important tool for metabolic engineering is ^{13}C-metabolic flux analysis. This technique provides highly accurate estimates of intracellular fluxes that could be used to pinpoint bottlenecks in the metabolic network that limit the production rates [61]. All of these established in silico techniques can be readily applied for enhancing the production of nutrients from cyanobacteria.

Conclusions

Synthetic biology has facilitated the rise of cyanobacteria as promising hosts for efficient conversion of solar energy to chemical energy, and the resulting product storage in the form of biomass or bioproducts. Recent progress in the optimization of photosynthesis and carbon fixation, identification of growth limitation factors, and isolation of fast growers has significantly advanced the potential large-scale production of cyanobacterial biomass. Meanwhile, engineering cyanobacteria to produce specific nutrients has emerged as an attractive complementary direction for further exploration. However, limiting factors for the wide application of cyanobacteria as model microbes remain the development of systematic and precise genetic tools and the elucidation of the relevant intracellular native regulations. Additionally, we need to be aware that 97% of tested cyanobacteria produce toxins [62,63], secondary metabolites posing a threat to human health, which should be considered in future studies.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Garcia-Pichel F, Lombard J, Soule T, Dunaj S, Wu SH, Wojciechowski MF: **Timing the evolutionary advent of cyanobacteria and the later great oxidation event using gene phylogenies of a sunscreen**. *mBio* 2019, **10**:e00561.

2. Singh SP, Pathak J, Sinha RP: **Cyanobacterial factories for the production of green energy and value-added products: an integrated approach for economic viability**. *Renew Sustain Energy Rev* 2017, **69**:578-595.

3. Linder T: **Making the case for edible microorganisms as an integral part of a more sustainable and resilient food production system**. *Food Sec* 2019:1-14.

4. Mullineaux CW, Liu LN: **Membrane dynamics in phototrophic bacteria**. *Annu Rev Microbiol* 2020, **74**.

5. Hill NC, Tay JW, Altus S, Bortz DM, Cameron JC: **Life cycle of a cyanobacterial carboxysome**. *Sci Adv* 2020, **6**:eaba1269.

6. Grosshagauer S, Kraemer K, Somoza V: **The true value of *Spirulina***. *J Agric Food Chem* 2020, **68**:4109-4115.

7. Gantar M, Svirčev Z: **Microalgae and cyanobacteria: food for thought**. *J Phycol* 2008, **44**:260-268.

8. Vanthoor-Koopmans M, Cordoba-Matson MV, Arredondo-Vega BO, Lozano-Ramírez C, Garcia-Trejo JF, Rodriguez-Palacio MC: **Microalgae and cyanobacteria production for feed and food supplements**. *Biosystems Engineering: Biofactories for Food Production in the Century XXI*. Springer; 2014:253-275.

9. Chittora D, Meena M, Barupal T, Swapnil P: **Cyanobacteria as a source of biofertilizers for sustainable agriculture**. *Biochem Biophys Rep* 2020, **22** 100737.

10. Karemore A, Yuan Y, Porubsky W, Chance R: **Biomass and pigment production for *Arthrospira platensis* via semi-continuous cultivation in photobioreactors: temperature effects**. *Biotechnol Bioeng* 2020, **117**:3081.

11. Sánchez-Bayo A, Morales V, Rodríguez R, Vicente G, Bautista LF: **Cultivation of microalgae and cyanobacteria: effect of operating conditions on growth and biomass composition**. *Molecules* 2020, **25**.

12. Jin SE, Lee SJ, Park CY: **Mass-production and biomarker-based characterization of high-value *Spirulina* powder for nutritional supplements**. *Food Chem* 2020, **325** 126751.

13. Pathak J, Maurya PK, Singh SP, Häder D-P, Sinha RP: **Cyanobacterial farming for environment friendly sustainable agriculture practices: innovations and perspectives**. *Front Environ Sci* 2018, **6**:7.

14. Sengupta A, Pakrasi HB, Wangikar PP: **Recent advances in synthetic biology of cyanobacteria**. *Appl Microbiol Biotechnol* 2018, **102**:5457-5471.

15. Ungerer J, Pakrasi HB: **Cpf1 Is a versatile tool for CRISPR genome editing across diverse species of cyanobacteria**. *Sci Rep* 2016, **6**:39681.

16. • Ho MY, Shen G, Canniffe DP, Zhao C, Bryant DA: **Light-dependent chlorophyll f synthase is a highly divergent paralog of PsbA of photosystem II**. *Science* 2016, **353**:1312-1317.
Chlorophyll f synthase was identified in this study in two cyanobacteria that are acclimatized to grow with far-red light. Furthermore, introduction of the chlorophyll f synthase encoding gene into *Synechococcus* 7002 successfully biosynthesized chlorophyll f in this model organism. This study expanded the range of light wavelength that phototroph can use for photosynthesis.

17. Tros M, Bersanini L, Shen G, Ho MY, van Stokkum IHM, Bryant DA, Croce R: **Harvesting far-red light: functional integration of chlorophyll *f* into Photosystem I complexes of *Synechococcus* Sp. PCC 7002**. *Biochim Biophys Acta Bioenerg* 2020, **1861** 148206.

18. Hasunuma T, Matsuda M, Senga Y, Aikawa S, Toyoshima M, Shimakawa G, Miyake C, Kondo A: **Overexpression of *flv3* improves photosynthesis in the cyanobacterium *Synechocystis* sp. PCC6803 by enhancement of alternative electron flow**. *Biotechnol Biofuels* 2014, **7**:493.

19. Liang F, Lindblad P: **Effects of overexpressing photosynthetic carbon flux control enzymes in the cyanobacterium *Synechocystis* PCC 6803**. *Metab Eng* 2016, **38**:56-64.

20. De Porcellinis AJ, Nørgaard H, Brey LMF, Erstad SM, Jones PR, Heazlewood JL, Sakuragi Y: **Overexpression of bifunctional fructose-1,6-bisphosphatase/sedoheptulose-1,7-bisphosphatase leads to enhanced photosynthesis and global reprogramming of carbon metabolism in *Synechococcus* sp. PCC 7002**. *Metab Eng* 2018, **47**:170-183.

21. Gupta JK, Rai P, Jain KK, Srivastava S: **Overexpression of bicarbonate transporters in the marine cyanobacterium *Synechococcus* sp. PCC 7002 increases growth rate and glycogen accumulation**. *Biotechnol Biofuels* 2020, **13**:17.

22. Kamennaya NA, Ahn S, Park H, Bartal R, Sasaki KA, Holman HY, Jansson C: **Installing extra bicarbonate transporters in the cyanobacterium *Synechocystis* sp. PCC6803 enhances biomass production**. *Metab Eng* 2015, **29**:76-85.

23. Yu J, Liberton M, Cliften PF, Head RD, Jacobs JM, Smith RD, Koppenaal DW, Brand JJ, Pakrasi HB: ***Synechococcus elongatus* UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO_2**. *Sci Rep* 2015, **5**:8132.

24. Jaiswal D, Sengupta A, Sohoni S, Sengupta S, Phadnavis AG, Pakrasi HB, Wangikar PP: **Genome features and biochemical characteristics of a robust, fast growing and naturally transformable cyanobacterium *Synechococcus elongatus* PCC 11801 isolated from India**. *Sci Rep* 2018, **8** 16632.

25. Jaiswal D, Sengupta A, Sengupta S, Madhu S, Pakrasi HB, Wangikar PP: **A novel cyanobacterium *Synechococcus elongatus* PCC 11802 has distinct genomic and metabolomic characteristics compared to its neighbor PCC 11801**. *Sci Rep* 2020, **10**:1-15.

26. Włodarczyk A, Selão TT, Norling B, Nixon PJ: **Newly discovered *Synechococcus* sp. PCC 11901 is a robust cyanobacterial strain for high biomass production**. *Commun Biol* 2020, **3**:215.

27. • Ungerer J, Lin PC, Chen HY, Pakrasi HB: **Adjustments to photosystem stoichiometry and electron transfer proteins are key to the remarkably fast growth of the cyanobacterium *Synechococcus elongatus* UTEX 2973**. *mBio* 2018, **9**:e02327.
This study systematically compared the photochemical and physiological phenotypes between UTEX 2973 and PCC 7942. It unveiled that faster growth rate corresponded to higher content of PSI, cytochrome b_6f, and plastocyanin, which lead to more oxidizing power to accept electrons and a higher flux of electrons transportation. This study provides a direction to improve photosynthesis efficiency in photosynthetic organisms.

28. •• Ungerer J, Wendt KE, Hendry JI, Maranas CD, Pakrasi HB: **Comparative genomics reveals the molecular determinants of rapid growth of the cyanobacterium *Synechococcus elongatus* UTEX 2973**. *Proc Natl Acad Sci U S A* 2018, **115**:E11761-E11770.
A comprehensive mutational analysis of UTEX 2973 was performed in this study and three genes, *atpA*, *ppnK*, and *rpaA*, were identified to confer the fast growth. More importantly, exchange of these genes in PCC 7942 to UTEX 2973 versions significantly reduced the doubling time from 6.8 hours to 2.3 hours. It revealed that the faster growing engineered strain had a higher photosynthetic productivity, which was caused by 5 SNPs substitution in threes genes related to energy metabolisms and circadian clock regulation. This milestone study revealed the molecular basis for fast growth and demonstrated that tiny genetic changes can significantly increase the growth rate of a microbe.

29. Van Alphen P, Abedini Najafabadi H, Branco Dos Santos F, Hellingwerf KJ: **Increasing the photoautotrophic growth rate of *Synechocystis* sp. PCC 6803 by identifying the limitations of its cultivation**. *Biotechnol J* 2018, **13**:e1700764.

30. Bernstein HC, McClure RS, Hill EA, Markillie LM, Chrisler WB, Romine MF, McDermott JE, Posewitz MC, Bryant DA, Konopka AE *et al.*: **Unlocking the constraints of cyanobacterial productivity: acclimations enabling ultrafast growth**. *mBio* 2016, **7**:e00949.

31. Mueller TJ, Ungerer JL, Pakrasi HB, Maranas CD: **Identifying the metabolic differences of a fast-growth phenotype in *Synechococcus* UTEX 2973**. *Sci Rep* 2017, **7**:41569.

32. Hendry JI, Gopalakrishnan S, Ungerer J, Pakrasi HB, Tang YJ, Maranas CD: **Genome-scale fluxome of *Synechococcus elongatus* UTEX 2973 using transient ^{13}C-labeling data**. *Plant Physiol* 2019, **179**:761-769.

33. • Reimers AM, Knoop H, Bockmayr A, Steuer R: **Cellular trade-offs and optimal resource allocation during cyanobacterial diurnal growth**. *Proc Natl Acad Sci U S A* 2017, **114**:E6457-E6465.
In this study, the authors use a dynamic resource allocation model of PCC 7942 to understand how cellular resources are allocated during the course of a full diurnal cycle. Sensitivity analysis on the model revealed that the major determinant of growth rate is the assumed fraction of the non-catalytic proteins in the total proteome.

34. Burnap RL: **Systems and photosystems: cellular limits of autotrophic productivity in cyanobacteria**. *Front Bioeng Biotechnol* 2015, **3**:1.

35. Chew KW, Yap JY, Show PL, Suan NH, Juan JC, Ling TC, Lee DJ, Chang JS: **Microalgae biorefinery: high value products perspectives**. *Bioresour Technol* 2017, **229**:53-62.

36. Bhunia B, Prasad Uday US, Oinam G, Mondal A, Bandyopadhyay TK, Tiwari ON: **Characterization, genetic regulation and production of cyanobacterial exopolysaccharides and its applicability for heavy metal removal**. *Carbohydr Polym* 2018, **179**:228-243.

37. Kirsch F, Luo Q, Lu X, Hagemann M: **Inactivation of invertase enhances sucrose production in the cyanobacterium *Synechocystis* sp. PCC 6803**. *Microbiology* 2018, **164**:1220-1228.

38. Vayenos D, Romanos GE, Papageorgiou GC, Stamatakis K: ***Synechococcus elongatus* PCC7942: a cyanobacterium cell factory for producing useful chemicals and fuels under abiotic stress conditions**. *Photosynth Res* 2020 http://dx.doi.org/10.1007/s11120-020-00747-6. Online ahead of print.

39. Lin PC, Zhang F, Pakrasi HB: **Enhanced production of sucrose in the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973**. *Sci Rep* 2020, **10**:390.

40. Kumar Saini D, Yadav D, Pabbi S, Chhabra D, Shukla P: **Phycobiliproteins from *Anabaena variabilis* CCC421 and its production enhancement strategies using combinatory evolutionary algorithm approach**. *Bioresour Technol* 2020, **309**:123347.

41. Korosh TC, Markley AL, Clark RL, McGinley LL, McMahon KD, Pfleger BF: **Engineering photosynthetic production of L-lysine**. *Metab Eng* 2017, **44**:273-283.

42. Brey LF, Włodarczyk AJ, Bang Thøfner JF, Burow M, Crocoll C, Nielsen I, Zygadlo Nielsen AJ, Jensen PE: **Metabolic engineering of *Synechocystis* sp. PCC 6803 for the production of aromatic amino acids and derived phenylpropanoids**. *Metab Eng* 2020, **57**:129-139.

43. Deshpande A, Vue J, Morgan J: **Combining random mutagenesis and metabolic engineering for enhanced tryptophan production in *Synechocystis* sp. strain PCC 6803**. *Appl Environ Microbiol* 2020, **86**.

44. •• Wang L, Chen L, Yang S, Tan X: **Photosynthetic conversion of carbon dioxide to oleochemicals by cyanobacteria: recent advances and future perspectives**. *Front Microbiol* 2020, **11**:634.

The authors summarized current knowledge on the metabolism of fatty acids and membrane lipids in cyanobacteria. They specifically summarized recent progress on photosynthetic production of fatty acids, fatty alcohols, fatty alk(a/e)nes, and fatty acid esters in genetically engineered cyanobacteria. Key challenges and possible solutions in the field were also discussed in this paper.

45. Poole LB, Parsonage D, Sergeant S, Miller LR, Lee J, Furdui CM, Chilton FH: **Acyl-lipid desaturases and Vipp1 cooperate in cyanobacteria to produce novel omega-3 PUFA-containing glycolipids**. *Biotechnol Biofuels* 2020, **13**:83.

46. Chen G, Qu S, Wang Q, Bian F, Peng Z, Zhang Y, Ge H, Yu J, Xuan N, Bi Y *et al.*: **Transgenic expression of delta-6 and delta-15 fatty acid desaturases enhances omega-3 polyunsaturated fatty acid accumulation in *Synechocystis* sp. PCC6803**. *Biotechnol Biofuels* 2014, **7**:32.

47. Santos-Merino M, Garcillán-Barcia MP, de la Cruz F: **Engineering the fatty acid synthesis pathway in *Synechococcus elongatus* PCC 7942 improves omega-3 fatty acid production**. *Biotechnol Biofuels* 2018, **11**:239.

48. Saini DK, Pabbi S, Shukla P: **Cyanobacterial pigments: perspectives and biotechnological approaches**. *Food Chem Toxicol* 2018, **120**:616-624.

49. Lagarde D, Beuf L, Vermaas W: **Increased production of zeaxanthin and other pigments by application of genetic engineering techniques to *Synechocystis* sp. strain PCC 6803**. *Appl Environ Microbiol* 2000, **66**:64-72.

50. Shimada N, Okuda Y, Maeda K, Umeno D, Takaichi S, Ikeuchi M: **Astaxanthin production in a model cyanobacterium *Synechocystis* sp. PCC 6803**. *J Gen Appl Microbiol* 2020, **66**:116-120.

51. • Diao J, Song X, Zhang L, Cui J, Chen L, Zhang W: **Tailoring cyanobacteria as a new platform for highly efficient synthesis of astaxanthin**. *Metab Eng* 2020, **61**:275-287.

This is a metabolic engineering study using cyanobacteria to produce astaxanthin, a high value carotenoid. The authors constructed a highly efficient astaxanthin anabolic pathway and improved its production by 500-fold through stepwise optimization of the biosynthetic pathway and rational rewiring of the intracellular metabolism.

52. Hasunuma T, Takaki A, Matsuda M, Kato Y, Vavricka CJ, Kondo A: **Single-stage astaxanthin production enhances the nonmevalonate pathway and photosynthetic central metabolism in *Synechococcus* sp. PCC 7002**. *ACS Synth Biol* 2019, **8**:2701-2709.

53. Hendry JI, Bandyopadhyay A, Srinivasan S, Pakrasi HB, Maranas CD: **Metabolic model guided strain design of cyanobacteria**. *Curr Opin Biotechnol* 2019, **64**:17-23.

54. Santos-Merino M, Singh AK, Ducat DC: **New applications of synthetic biology tools for cyanobacterial metabolic engineering**. *Front Bioeng Biotechnol* 2019, **7**:33.

55. Klanchui A, Dulsawat S, Chaloemngam K, Cheevadhanarak S, Prommeenate P, Meechai A: **An improved genome-scale metabolic model of *Arthrospira platensis* C1 (iAK888) and its application in glycogen overproduction**. *Metabolites* 2018, **8**:84.

56. Broddrick JT, Rubin BE, Welkie DG, Du N, Mih N, Diamond S, Lee JJ, Golden SS, Palsson BO: **Unique attributes of cyanobacterial metabolism revealed by improved genome-scale metabolic modeling and essential gene analysis**. *Proc Natl Acad Sci U S A* 2016, **113**:E8344-E8353.

57. Segrè D, Vitkup D, Church GM: **Analysis of optimality in natural and perturbed metabolic networks**. *Proc Natl Acad Sci U S A* 2002, **99**:15112-15117.

58. Ranganathan S, Suthers PF, Maranas CD: **OptForce: an optimization procedure for identifying all genetic manipulations leading to targeted overproductions**. *PLoS Comput Biol* 2010, **6**:e1000744.

59. Nishiguchi H, Hiasa N, Uebayashi K, Liao J, Shimizu H, Matsuda F: **Transomics data-driven, ensemble kinetic modeling for system-level understanding and engineering of the cyanobacteria central metabolism**. *Metab Eng* 2019, **52**:273-283.

60. Wang X, Liu W, Xin C, Zheng Y, Cheng Y, Sun S, Li R, Zhu XG, Dai SY, Rentzepis PM *et al.*: **Enhanced limonene production in cyanobacteria reveals photosynthesis limitations**. *Proc Natl Acad Sci U S A* 2016, **113**:14225-14230.

61. McAtee AG, Jazmin LJ, Young JD: **Application of isotope labeling experiments and ^{13}C flux analysis to enable rational pathway engineering**. *Curr Opin Biotechnol* 2015, **36**:50-56.

62. Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B: **Diverse taxa of cyanobacteria produce beta-N-methylamino-L-alanine, a neurotoxic amino acid**. *Proc Natl Acad Sci U S A* 2005, **102**:5074-5078.

63. Du X, Liu H, Yuan L, Wang Y, Ma Y, Wang R, Chen X, Losiewicz MD, Guo H, Zhang H: **The diversity of cyanobacterial toxins on structural characterization, distribution and identification: a systematic review**. *Toxins (Basel)* 2019, **11**:530.