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COMMENTARY

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Metabolic pathway rewiring in engineered cyanobacteria for solar-to-chemical and solar-to-fuel production from CO_2

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ABSTRACT
Photoautotrophic cyanobacteria have been developed to convert CO_2 to valuable chemicals and fuels as solar-to-chemical (S2C) and solar-to-fuel (S2F) platforms. Here, I describe the rewiring of the metabolic pathways in cyanobacteria to better understand the endogenous carbon flux and to enhance the yield of heterologous products. The plasticity of the cyanobacterial metabolism has been proposed to be advantageous for the development of S2C and S2F processes. The rewiring of the sugar catabolism and of the phosphoketolase pathway in the central cyanobacterial metabolism allowed for an enhancement in the level of target products by redirecting the carbon fluxes. Thus, metabolic pathway rewiring can promote the development of more efficient cyanobacterial cell factories for the generation of feasible S2C and S2F platforms.

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Introduction

Global concerns targeting the reduction of greenhouse gas emissions and sustaining the supply of energy and chemicals have brought attention to the development of sustainable platforms to convert carbon dioxide to chemicals and fuels, in the form of solar-to-chemical (S2C) and solar-to-fuel (S2F) technologies.[1] The S2C and S2F platforms have been developed to produce the desired value-added chemicals and fuels from 3 elements (CO_2, H_2O, and solar energy). Together with the development of integrated bio-electrochemical systems[2,3] based on engineered lithoautotrophic bacteria for sustainable S2C and S2F production, photoautotrophic cyanobacteria have also been genetically engineered as S2C and S2F platforms to directly produce value-added chemicals from CO_2. Recent reviews on the development of S2C and S2F platforms using engineered cyanobacteria have focused on general perspectives for cyanobacterial fuels (Cyanofuels),[4] on the engineering of metabolic pathways in cyanobacteria,[5-7] on the coupling of enzymes to the photosynthetic reducing power,[8] and discussed future perspectives from a systems biology point of view.[9] Thus, I have provided a more detailed analysis of the rewiring of metabolic pathways to increase the carbon flux of CO_2 toward target end products.

Metabolic pathway rewiring to improve carbon assimilation

To achieve the production of final products at a feasible scale, product yield and productivity must be considered under both light and dark conditions. The implementation of the sugar utilization pathway in cyanobacteria has successfully increased the product yield under either continuous or diurnal conditions.[10,11] The heterologous expression of the galactose (GalP) or xylose (XylE) transporters, of xylose isomerase (XylA), and xylulokinase (XylB) from *E. coli* has resulted in the enhanced production of 2,3-butanediol (2,3-BDO), beside CO_2 fixation, in cyanobacteria supplemented with glucose or xylose. Subsequently, metabolite profiling analysis was performed to assess the ratio of carbon assimilation from sugar and CO_2 through the feeding of labeled [U-^{13}C] glucose. Recently, the co-utilization of glucose and CO_2 has been optimized to improve 2,3-BDO production and yield, by guiding the glucose flux into the central metabolism.[12] Overexpression of key genes such as *zwf* (encoding for a glucose-6-phosphate dehydrogenase) and *gnd* (encoding for a 6-phosphogluconate dehydrogenase) in the oxidative pentose phosphate pathway, *prk* (encoding for a phosphoribulokinase), and *rbcLXS* (encoding for a ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) subunit) led to the rewiring of

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glucose catabolism and the fixation of CO_2, and increased the growth rate, glucose consumption, and 2,3-BDO production (1.1 g/L/d) in cyanobacteria. Photomixotrophic production using engineered cyanobacteria via the rewiring of metabolic pathways could be advantageous for high cell-density cultivation to increase the production yield, despite some concerns regarding the cost and the contamination of the sugar feedstock. In addition, D-lactic acid (2.17 g/L)[13] and ethylene (821 ± 52 μL/L/h)[14] have been photo-mixotrophically produced in engineered cyanobacteria using either acetate or xylose as additional carbon sources, respectively.

Metabolic pathway rewiring to supply a key intermediate for improving production

Metabolic flux analysis[15] and flux balance analysis[16] have been performed in cyanobacteria to determine the carbon fluxes from CO_2 and sugars, and to assist in the metabolic engineering of cyanobacteria. A relatively small fraction of carbon fluxes from CO_2 were directed to fatty acids and the terpenoid biosynthesis pathway.[17] Although the regulation of carbon partitioning in the cyanobacterial cell is not fully understood, it can be flexibly altered under certain conditions, such as nutrient deprivation and irradiance stress. Thus, it is necessary to perform pathway engineering in cyanobacteria to redirect carbon fluxes to the final product, in addition to reconstructing the metabolic pathway for the target product. Recent studies have addressed metabolic pathway rewiring to increase product yields by enhancing intermediate pools. For example, the engineered *Synechococcus elongatus* PCC 7942, a model cyanobacterium, harboring heterologous genes for acetone biosynthesis, did not produce any acetone from CO_2 under light conditions.[18] Subsequently, modular pathway engineering in *S. elongatus* PCC 7942 through the rewiring of the phosphoketolase (PHK) pathway to the acetone biosynthesis pathway has allowed the production of photosynthetic acetone from CO_2 (Fig. 1). The rewired PHK pathway increased the level of the intermediate pool of acetyl-CoA that was used for improving acetone production. Consistently, the PHK pathway has been successfully rewired to the central metabolism of cyanobacteria to enhance the production levels of *n*-butanol[19] and fatty acid ethyl esters,[20] respectively. Another benefit of rewiring the PHK pathway is that the engineered cyanobacteria used for S2C and S2F platforms can be carbon efficient by bypassing pyruvate decarboxylation.[21,22]

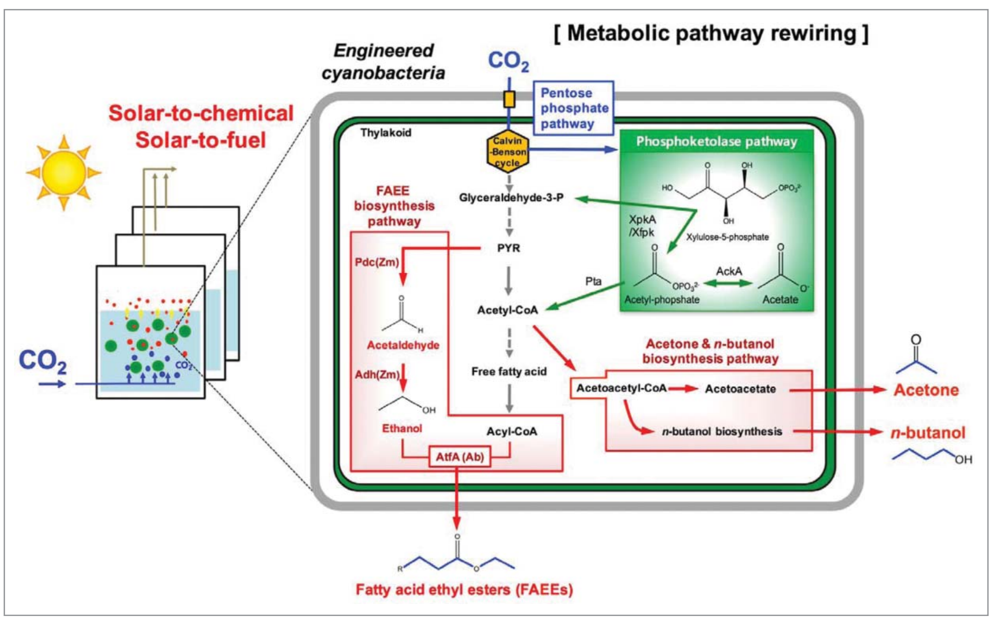

Figure 1. Development of engineered cyanobacteria through metabolic rewiring to construct solar-to-chemical and solar-to-fuel platforms. The rewiring of the heterologous phosphoketolase pathway to the pentose phosphate pathway in cyanobacteria has enhanced the levels of the acetyl-coA pool, resulting in an increased production of acetone,[18] *n*-butanol,[19] and fatty acid ethyl esters (FAEEs)[20] from CO_2. The phosphoketolase pathway is shown in the green box and the heterologous chemical-producing pathways are shown in red boxes. The carbon flux of CO_2 is indicated by the blue arrow, and the carbon backbone that originated from CO_2 is also shown in blue. XpkA/Xfpk, phosphoketolase; AckA, acetate kinase; Pta, phosphotransacetylase; Pdc (Zm), Pyruvate decarboxylase of *Zymomonas mobilis*; Adh (Zm), Aldehyde dehydrogenase of *Z. mobilis*.

The plasticity of the cyanobacterial metabolism in the rewiring of metabolic pathways

The cyanobacterial metabolism is complex and plastic. This is supported by genetic and biochemical evidence that demonstrates the presence of 2-oxoglutarate decarboxylase and succinate semialdehyde dehydrogenase activities in the tricarboxylic acid cycle (TCA),[23] the presence of the Entner-Doudoroff pathway,[24] the glyoxylate cycle,[25] and the gamma-aminobutyric acid shunt.[26] Moreover, kinetic profiling of isotope-labeled metabolites has uncovered that the functional PHK pathway in *Synechocystis* sp. PCC 6803 is flexible, and has the potential to increase the efficiency of carbon metabolism and photosynthetic productivity.[27] Independently, the engineering of cyanobacteria for the production of ethylene has revealed the plasticity of carbon metabolism by redirecting 37% of the fixed carbon flux into the TCA cycle, and by increasing the photosynthetic productivity for ethylene (718 ± 19 μL/L/h/OD_{730}).[28]

Metabolic plasticity is also associated with the metabolic capabilities under various environmental growth conditions (e.g., photoautotrophic, photomixotrophic, or heterotrophic growth),[29,30] and this has been demonstrated through the rewiring of metabolic pathways for the production of 2,3-BDO.[12] Thus, the rewiring of metabolic pathways for directing carbon fluxes toward the desired products in cyanobacteria could facilitate the development of feasible S2C and S2F platforms.

Conclusion

The status of solar-to-chemical and solar-to-fuel platforms for the production of value-added chemicals from CO_2 has been addressed by focusing on the metabolic engineering of cyanobacteria. The rewiring of the metabolic pathways in cyanobacteria has allowed for the production of non-native chemicals, and facilitated carbon partitioning toward target chemicals using the S2C and S2F platforms. In addition, protein engineering[31] and CRISPR-Cas9 genetic tools[32] for metabolic engineering will likely promote the development of more efficient cyanobacterial cell factories. Moreover, photo-bioprocess engineering will be used for the generation of feasible S2C and S2F platforms.

Disclosure of potential conflicts of interest

The author declares no potential conflicts of interest.

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References

[1] Woo HM. Solar-to-chemical and solar-to-fuel production from CO2 by metabolically engineered microorganisms. Curr Opin Biotechnol 2017; 45:1-7; https://doi.org/10.1016/j.copbio.2016.11.017

[2] Torella JP, Gagliardi CJ, Chen JS, Bediako DK, Colon B, Way JC, Silver PA, Nocera DG. Efficient solar-to-fuels production from a hybrid microbial-water-splitting catalyst system. Proc Natl Acad Sci U S A 2015; 112:2337-42; PMID:25675518; https://doi.org/10.1073/pnas.1424872112

[3] Nichols EM, Gallagher JJ, Liu C, Su Y, Resasco J, Yu Y, Sun Y, Yang P, Chang MC, Chang CJ. Hybrid bioinorganic approach to solar-to-chemical conversion. Proc Natl Acad Sci U S A 2015; 112:11461-6; PMID:26305947; https://doi.org/10.1073/pnas.1508075112

[4] Sarsekeyeva F, Zayadan BK, Usserbaeva A, Bedbenov VS, Sinetova MA, Los DA. Cyanofuels: biofuels from cyanobacteria. Reality and perspectives. Photosynth Res 2015; 125:329-40; https://doi.org/10.1007/s11120-015-0103-3

[5] Case AE, Atsumi S. Cyanobacterial chemical production. J Biotechnol 2016; 231:106-14; https://doi.org/10.1016/j.jbiotec.2016.05.023

[6] Savakis P, Hellingwerf KJ. Engineering cyanobacteria for direct biofuel production from CO2. Curr Opin Biotechnol 2015; 33:8-14; https://doi.org/10.1016/j.copbio.2014.09.007

[7] Lindberg P, Park S, Melis A. Engineering a platform for photosynthetic isoprene production in cyanobacteria, using Synechocystis as the model organism. Metab Eng 2010; 12:70-9; https://doi.org/10.1016/j.ymben.2009.10.001

[8] Mellor SB, Vavitsas K, Nielsen AZ, Jensen PE. Photosynthetic fuel for heterologous enzymes: the role of electron carrier proteins. Photosynth Res 2017; PMID:28285375; https://doi.org/10.1007/s11120-017-0364-0

[9] Gudmundsson S, Nogales J. Cyanobacteria as photosynthetic biocatalysts: a systems biology perspective. Mol Biosyst 2015; 11:60-70; PMID:25382198; https://doi.org/10.1039/C4MB00335G

[10] McEwen JT, Kanno M, Atsumi S. 2,3 Butanediol production in an obligate photoautotrophic cyanobacterium in dark conditions via diverse sugar consumption. Metab Eng 2016; 36:28-36; https://doi.org/10.1016/j.ymben.2016.03.004

[11] McEwen JT, Machado IM, Connor MR, Atsumi S. Engineering *Synechococcus elongatus* PCC 7942 for continuous growth under diurnal conditions. Appl Environ Microbiol 2013; 79:1668-75; PMID:23275509; https://doi.org/10.1128/AEM.03326-12

[12] Kanno M, Carroll AL, Atsumi S. Global metabolic rewiring for improved CO2 fixation and chemical production in cyanobacteria. Nat Commun 2017; 8:14724; PMID:28287087; https://doi.org/10.1038/ncomms14724

[13] Varman AM, Yu Y, You L, Tang YJ. Photoautotrophic production of D-lactic acid in an engineered cyanobacterium. Microb Cell Fact 2013; 12:117; PMID:24274114; https://doi.org/10.1186/1475-2859-12-117

[14] Lee TC, Xiong W, Paddock T, Carrieri D, Chang IF, Chiu HF, Ungerer J, Juo SH, Maness PC, Yu J. Engineered xylose utilization enhances bio-products productivity in the cyanobacterium *Synechocystis* sp. PCC 6803. Metab Eng 2015; 30:179-89; PMID:26079651; https://doi.org/10.1016/j.ymben.2015.06.002

[15] Young JD, Shastri AA, Stephanopoulos G, Morgan JA. Mapping photoautotrophic metabolism with isotopically nonstationary (13)C flux analysis. Metab Eng 2011; 13:656-65; https://doi.org/10.1016/j.ymben.2011.08.002

[16] Shirai T, Osanai T, Kondo A. Designing intracellular metabolism for production of target compounds by introducing a heterologous metabolic reaction based on a *Synechosystis* sp 6803 genome-scale model. Microbial Cell Factories 2016; 15:13. doi:ARTN 13; PMID:26783098; https://doi.org/10.1186/s12934-016-0416-8

[17] Melis A. Carbon partitioning in photosynthesis. Curr Opin Chem Biol 2013; 17:453-6; https://doi.org/10.1016/j.cbpa.2013.03.010

[18] Chwa JW, Kim WJ, Sim SJ, Um Y, Woo HM. Engineering of a modular and synthetic phosphoketolase pathway for photosynthetic production of acetone from CO in *Synechococcus elongatus* PCC 7942 under light and aerobic condition. Plant Biotechnol J 2016; 14:1768-76; PMID:26879003; https://doi.org/10.1111/pbi.12536

[19] Anfelt J, Kaczmarzyk D, Shabestary K, Renberg B, Rockberg J, Nielsen J, Uhlén M, Hudson EP. Genetic and nutrient modulation of acetyl-CoA levels in *Synechocystis* for n-butanol production. Microb Cell Fact 2015; 14:167; PMID:26474754; https://doi.org/10.1186/s12934-015-0355-9

[20] Lee HJ, Choi J, Lee S-M, Um Y, Sim SJ, Kim Y, Woo HM. Photosynthetic CO2 Conversion to Fatty Acid Ethyl Esters (FAEEs) Using Engineered Cyanobacteria. J Agric Food Chem 2017; 65:1087-92; PMID:28128561; https://doi.org/10.1021/acs.jafc.7b00002

[21] Henard CA, Freed EF, Guarnieri MT. Phosphoketolase pathway engineering for carbon-efficient biocatalysis. Curr Opin Biotechnol 2015; 36:183-8; https://doi.org/10.1016/j.copbio.2015.08.018

[22] Bogorad IW, Lin TS, Liao JC. Synthetic non-oxidative glycolysis enables complete carbon conservation. Nature 2013; 502:693-7; PMID:24077099; https://doi.org/10.1038/nature12575

[23] Zhang S, Bryant DA. The tricarboxylic acid cycle in cyanobacteria. Science 2011; 334:1551-3; PMID:22174252; https://doi.org/10.1126/science.1210858

[24] Chen X, Schreiber K, Appel J, Makowka A, Fahnrich B, Roettger M, Hajirezaei MR, Sönnichsen FD, Schönheit P, Martin WF, et al. The Entner-Doudoroff pathway is an overlooked glycolytic route in cyanobacteria and plants. Proc Natl Acad Sci U S A 2016; 113:5441-6; PMID:27114545; https://doi.org/10.1073/pnas.1521916113

[25] Zhang SY, Bryant DA. Biochemical Validation of the Glyoxylate Cycle in the Cyanobacterium *Chlorogloeopsis fritschii* Strain PCC 9212. J Biol Chem 2015; 290:14019-30; PMID:25869135; https://doi.org/10.1074/jbc.M115.648170

[26] Xiong W, Brune D, Vermaas WF. The gamma-aminobutyric acid shunt contributes to closing the tricarboxylic acid cycle in *Synechocystis* sp. PCC 6803. Mol Microbiol 2014; 93:786-96; PMID:24989231; https://doi.org/10.1111/mmi.12699

[27] Xiong W, Lee TC, Rommelfanger S, Gjersing E, Cano M, Maness PC, Ghirardi M, Yu J. Phosphoketolase pathway contributes to carbon metabolism in cyanobacteria. Nat Plants 2015; 2:15187; PMID:27250745; https://doi.org/10.1038/nplants.2015.187

[28] Xiong W, Morgan JA, Ungerer J, Wang B, Maness PC, Yu JP. The plasticity of cyanobacterial metabolism supports direct CO2 conversion to ethylene. Nat Plants 2015; 1:15053. doi:Artn 15053; https://doi.org/10.1038/nplants.2015.53

[29] Wan N, DeLorenzo DM, He L, You L, Immethun CM, Wang G, Baidoo EE, Hollinshead W, Keasling JD, Moon TS, et al. Cyanobacterial carbon metabolism: Fluxome plasticity and oxygen dependence. Biotechnol Bioeng 2017; https://doi.org/10.1002/bit.26287

[30] Nakajima T, Kajihata S, Yoshikawa K, Matsuda F, Furusawa C, Hirasawa T, Shimizu H. Integrated metabolic flux and omics analysis of *Synechocystis* sp. PCC 6803 under mixotrophic and photoheterotrophic conditions. Plant Cell Physiol 2014; 55:1605-12; PMID:24969233; https://doi.org/10.1093/pcp/pcu091

[31] Formighieri C, Melis A. A phycocyanin.phellandrene synthase fusion enhances recombinant protein expression and beta-phellandrene (monoterpene) hydrocarbons production in *Synechocystis* (cyanobacteria). Metab Eng 2015; 32:116-24; https://doi.org/10.1016/j.ymben.2015.09.010

[32] Gordon GC, Korosh TC, Cameron JC, Markley AL, Begemann MB, Pfleger BF. CRISPR interference as a titratable, trans-acting regulatory tool for metabolic engineering in the cyanobacterium *Synechococcus* sp. strain PCC 7002. Metab Eng 2016; 38:170-9; https://doi.org/10.1016/j.ymben.2016.07.007