Metabolic pathway rewiring in engineered cyanobacteria for solar-to-chemical and solar-to-fuel production from CO₂

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ABSTRACT
Photoautotrophic cyanobacteria have been developed to convert CO₂ 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.

KEYWORDS
cyanobacteria; metabolic engineering; metabolic pathway rewiring; solar fuel; solar-to-chemical

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. The S₂C and S₂F platforms have been developed to produce the desired value-added chemicals and fuels from 3 elements (CO₂, H₂O, and solar energy). Together with the development of integrated bio-electrochemical systems 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 CO₂. Recent reviews on the development of S₂C and S₂F platforms using engineered cyanobacteria have focused on general perspectives for cyanobacterial fuels (Cyanofuels), on the engineering of metabolic pathways in cyanobacteria, on the coupling of enzymes to the photosynthetic reducing power, and discussed future perspectives from a systems biology point of view. Thus, I have provided a more detailed analysis of the rewiring of metabolic pathways to increase the carbon flux of CO₂ 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. 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 CO₂ fixation, in cyanobacteria supplemented with glucose or xylose. Subsequently, metabolite profiling analysis was performed to assess the ratio of carbon assimilation from sugar and CO₂ through the feeding of labeled [U-¹³C] glucose. Recently, the co-utilization of glucose and CO₂ has been optimized to improve 2,3-BDO production and yield, by guiding the glucose flux into the central metabolism. 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...
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)\textsuperscript{13} and ethylene (821 ± 52 μL/L/h)\textsuperscript{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\textsuperscript{15} and flux balance analysis\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{19} and fatty acid ethyl esters,\textsuperscript{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.\textsuperscript{21,22}

![Diagram of metabolic pathway rewiring](image-url)
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), the presence of the Entner-Doudoroff pathway, the glyoxylate cycle, and the gamma-aminobutyric acid shunt. 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. 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/OD730). Metabolic plasticity is also associated with the metabolic capabilities under various environmental growth conditions (e.g., photoautotrophic, photomixotrophic, or heterotrophic growth), and this has been demonstrated through the rewiring of metabolic pathways for the production of 2,3-BDO. 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 CO2 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 and CRISPR-Cas9 genetic tools 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.

**Funding**

This work was supported by the Korean CCS R&D Center (KCRC) (2014M1A8A1049277) and Basic Science Research Program (2017R1A2B2002566) through the National Research Foundation of Korea, funded by the Korean Government. In addition, this work is partially supported by the Golden Seed Project (213008–05–1-WT911) grant funded by the Ministry of Agriculture, Ministry of the Oceans and Fisheries.

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