Protein Cell 2010, 1(3): 207–210
DOI 10.1007/s13238-010-0043-9

Protein & Cell

PERSPECTIVE

# Engineering cyanobacteria for fuels and chemicals production

**Jie Zhou, Yin Li** ✉

Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
✉ Correspondence: yli@im.ac.cn

**The world's energy and global warming crises call for sustainable, renewable, carbon-neutral alternatives to replace fossil fuel resources. Currently, most biofuels are produced from agricultural crops and residues, which lead to concerns about food security and land shortage. Compared to the current biofuel production system, cyanobacteria, as autotrophic prokaryotes, do not require arable land and can grow to high densities by efficiently using solar energy, $CO_2$, water, and inorganic nutrients. Moreover, powerful genetic techniques of cyanobacteria have been developed. For these reasons, cyanobacteria, which carry out oxygenic photosynthesis, are attractive hosts for production of fuels and chemicals. Recently, several chemicals including ethanol, isobutanol and isoprene have been produced by engineered cyanobacteria directly using solar energy, $CO_2$, and water. Cyanobacterium is therefore a potential novel cell factory for fuels and chemicals production to address global energy security and climate change issues.**

Energy is the basis for the rapid development of modern industry and economy. In 2008, 13 terrawatts (1 TW = $10^{12}$ W = 3.2 EJ/year) of energy was consumed worldwide, of which approximately 80% was generated from burning fossil materials (Rittmann, 2008). As a consequence, about 6 gigatons (Gton = $10^9$ tons) $CO_2$ was produced from combustion of fossil fuels annually (Rittmann, 2008). In consideration of the energy security and environmental concerns, production of fuels and chemicals from renewable carbon resources has gained increasing attention, with the aim to reduce the dependence on fossil resources. According to the Renewable Fuels Association, 80 billion liters of biofuels were produced worldwide in 2009. Currently, most of the biofuels are produced from agricultural crops, such as bioethanol from corn and biodiesel from soybeans. Statistics from the US Department of Agriculture indicate that 25% of total food production in the US was used to produce biofuels. With the increasing demand of biofuels, the negative impacts of crop-based biofuels are becoming more evident. This includes the impact on food prices and the enhancing demands for arable land and fresh water. Therefore, it becomes increasingly urgent to develop novel approaches so that biofuels and chemicals can be produced from sustainable, renewable, carbon-neutral alternatives to replace crop-based biofuels.

Cyanobacteria, also known as blue-green algae, have recently gained great attention because of their potential application in biofuels and chemicals production. Cyanobacteria are photoautotrophic prokaryotes, which can convert solar energy into chemical energy and fix $CO_2$ via photosynthesis. Although cyanobacteria carry out oxygenic photosynthesis that is similar to that in higher plants, the photosynthetic efficiency of cyanobacteria is much higher than that of higher plants. A previous review has shown that microalgae can convert 10%–20% of solar energy into chemicals while higher plants can only convert 0.5% (Li et al., 2008). In addition, compared to higher plant, cyanobacteria can tolerate higher $CO_2$ content in gas streams, which might provide a novel approach to convert $CO_2$ from the flue gases into organic substances. The utilization of cyanobacterium *Anabaena* sp. in $CO_2$ removal from flue gases has been evaluated (GonzálezLópez et al., 2009). As autotrophic prokaryotes, cyanobacteria have simple growth requirement and can use light, $CO_2$, and other inorganic nutrients efficiently to grow to high cell densities. Cyanobacteria exhibit diversity in metabolism and habitat; some species can even thrive in salt water. Moreover, cyanobacteria can complete more than one entire growing cycle in one day. Actually, the biomass doubling time during exponential growth phase for some cyanobacteria species including *Synechocystis* sp., *Synechococcus* sp. and *Anabaena* sp. is as short as several hours. Furthermore, powerful genetic tools for some cyanobacteria species have been well

developed. Therefore, cyanobacteria are promising sustainable hosts for biofuels and chemicals production because of high solar energy conversion efficiency, high $CO_2$ tolerance, rapid growth rate, low demand for arable land and fresh water, and more amenable to genetic engineering than eukaryotic microalgae. Engineered photosynthetic cyanobacteria can be used as a novel cell factory for efficient biofuels and chemicals production, in which conversion of solar energy, carbon sequestration and biofuels and chemicals production are combined.

## ETHANOL PRODUCTION

Currently, bioethanol, one of the major biofuels, is mainly produced from the fermentation of agricultural crops including corn, sorghum and sugarcane. Concerns over limited arable land, food shortage and the cost competiveness of production processes have significantly hampered further development of crop-based ethanol production. Alternative bioethanol production approaches need to be developed to address the above problems. A pathway for ethanol biosynthesis was successfully introduced into cyanobacterium *Synechococcus* sp. 7942 by expressing two enzymes, pyruvate decarboxylase and alcohol dehydrogenase, via a shuttle vector (Deng and Coleman, 1999). Although the ethanol productivity from the engineered *Synechococcus* was quite low as compared to that of the yeast fermentation, it proves the concept that ethanol can be produced directly from solar energy, $CO_2$ and water using engineered cyanobacteria. From then on, optimization of the ethanol production system is being performed. This includes optimization of growth conditions and further genetic improvement of host cells. The ethanol yield of the engineered cyanobacteria has increased from 54 nmol /$OD_{730}$ unit/ liter/day to 5.2 mmol/$OD_{730}$ unit/liter/day by another genetic manipulation approach (Dexter and Fu, 2009). Furthermore, Algenol Biofuels Company in Naples, Florida, which produces ethanol directly from solar energy and carbon dioxide using metabolically engineered cyanobacteria, estimates that its technology will produce 10,000–12,000 gal/ac/yr of ethanol which is much higher than that of crop-based ethanol (Waltz, 2009).

## ISOBUTANOL PRODUCTION

Butanol is an important chemical that is widely used in petrochemical industry. Moreover, compared to ethanol, butanol is considered one of the advanced biofuels because of higher energy density, lower hygroscopicity, lower vapor pressure and less corrosiveness (Atsumi and Liao, 2008). Currently, the production of butanol is from the conventional acetone-butanol-ethanol fermentation pathway of *Clostridium acetobutylicum* or via keto-acid pathways of the engineered *E. coli* (Atsumi et al., 2008) using sugars as raw materials. To reduce $CO_2$ emissions and alleviate food shortage, Atsumi et al. genetically engineered photoautotrophic prokaryote, cyanobacterium *Synechococcus* elongates 7942, to produce isobutanol directly from $CO_2$ and solar energy (Atsumi et al., 2009). This was achieved by introducing the isobutanol synthetic pathway and a key enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) involved in $CO_2$ fixation pathway, into cyanobacterium. The engineered cyanobacterium strain achieved high productivity of isobutanol, which is competitive with the current corn and cellulosic ethanol technology (Atsumi et al., 2009; Sheehan, 2009).

## ISOPRENE PRODUCTION

Isoprene ($C_5H_8$) is a useful feedstock in the synthetic chemistry industry. In addition, isoprene is also considered one of the advanced biofuels because it stores more energy and is not miscible in water as compared to ethanol (Atsumi and Liao, 2008; Lindberg et al., 2010). Isoprene is a natural product of a variety of herbaceous plants and is released into the surrounding environments. However, these plants are unsuitable for production of isoprene due to the volatility of isoprene and low solar energy conversion efficiency (Lindberg et al., 2010; Melis, 2009). Production of isoprene by engineered heterotrophic bacterium *E. coli* for has been reviewed (Atsumi and Liao, 2008; Fortman et al., 2008). Recently, Lindberg et al. (2010) developed a novel approach for production of isoprene in engineered photosynthetic prokaryote, cyanobacterium *Synechocystis*, by heterologous expression of isoprene synthase gene. Although the titer of isoprene produced from the engineered cyanobacterium strain is much lower as compared to the engineered *E. coli*, the importance and novelty of this strategy is that isoprene can be directly produced from $CO_2$ and solar energy, which might significantly reduce the production cost.

## PROSPECTIVES

Genetically engineered cyanobacteria provide a promising system for both biofuel and chemical production and $CO_2$ utilization. Although further improvement is required, the approach of producing biofuels and chemicals from cyanobacteria offers potential unprecedented advantages over that of the crop-based production system. To make this novel approach economically feasible, further improvements are needed. This includes the solar energy conversion efficiency, large-scale cultivation techniques, and tolerance to the target product. In addition, production of high-value products in association with the production of biofuels and chemicals might also improve the economic feasibility of the cyanobacterial production system (Li et al., 2008).

### Improving photosynthesis efficiency

Photosynthetic capability is a promising target for further increasing the productivity of biofuels and chemical from engineered cyanobacteria. Oxygenic photosynthesis is limited

 

by multiple factors including photosynthetic electron transport, regeneration of $CO_2$ acceptor molecules in the Calvin cycle, the activity and substrate specificity of Rubisco, and photorespiratory (Peterhansel et al., 2008). Under plenty $CO_2$ supply and sufficient illumination conditions, Rubisco is the main limited factor for carbon fixation. The activity of Rubisco is very low, which can only fix a few $CO_2$ molecules per second (Portis and Parry, 2007). Therefore, huge quantities of Rubisco are required to fix $CO_2$ in photosynthetic organism. In an effort to produce isobutanol directly from $CO_2$, Rubisco was overexpressed in the cyanobacterium to increase the photosynthetic capability. Although Rubisco overexpression did not increase the photosynthetic rate, the productivity of isobutanol was significant increased, which means overexpression of Rubisco can increase the efficiency of $CO_2$ fixation (Atsumi et al., 2009; Sheehan, 2009). To further increase $CO_2$ fixation efficiency, improvement of the activity and $CO_2$ substrate specificity of Rubisco will be a promising approach, although optimizing Rubisco enzyme by mutagenesis has received little success to date. Besides Rubisco, $CO_2$ fixation is also often limited by the regeneration of $CO_2$ accepter ribulose-1,5-bisphosphate in Calvin cycle of photosynthesis. Other potential approaches for improving photosynthetic efficiency have been excellently reviewed by Peterhansel et al. (2008).

### Optimizing large-scale cultivation

Large-scale cultivation is another key aspect that significantly affects the cost competiveness of the cyanobacterial production process. This topic has been discussed extensively in previous reviews (Chisti, 2008; Li et al., 2008). To minimize expense, the ideal cyanobacteria culture system should use freely available sunlight and open ponds. Concerning about the containments in open ponds culture system, many different designs of photobioreactor have been developed. Tubular photobioreactor, which has been successfully used in several microalgae companies worldwide, seems to be an ideal large-scale culture system for the production of biofuels and chemicals (Spolaore et al., 2006; Chisti, 2008). In addition, many improvements are being made in large-scale cultivation of cyanobacteria with respect to reducing cost, increasing operation stability and efficiency.

### Increasing cellular resistance to target products

The target products of these metabolically engineered cyanobacteria, such as ethanol and butanol, are toxic to the hosts and cyanobacteria strains are usually not able to withstand high concentrations of such products. Therefore, the cellular resistance of cyanobacteria to such products will determine the final titer that can be produced, which will have a great impact on productivity and product recovery of the target product. Adaptation, random mutagenesis, and introduction of a new resistance system will be potential approaches to increase the tolerance of cyanobacteria to such products (Ramos et al., 2002; Angermayr et al., 2009), and robustness to the environmental stresses (Zhang et al. 2009). In addition, timely collecting the products from cultivation system by using integrated production-recovery techniques will be important approaches to address this issue.

In conclusion, with rapid development of synthetic biology approaches, understandings of the physiology of cyanobacteria, and technologies for advanced photobioreactor design and downstream processing, production of biofuels and chemicals by engineered cyanobacteria system will have great potential in addressing the global energy and climate change issues, and thus will receive more attentions in the near future.

## ACKNOWLEDGEMENTS

This work was supported by Beijing Natural Science Foundation (Grant no. 5102026).

## REFERENCES

Angermayr, S.A., Hellingwerf, K.J., Lindblad, P., and Teixeira de Mattos de Mattos, M.J. (2009). Energy biotechnology with cyanobacteria. Curr Opin Biotechnol 20, 257–263.

Atsumi, S., and Liao, J.C. (2008). Metabolic engineering for advanced biofuels production from Escherichia coli. Curr Opin Biotechnol 19, 414–419.

Atsumi, S., Hanai, T., and Liao, J.C. (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451, 86–89.

Atsumi, S., Higashide, W., and Liao, J.C. (2009). Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat Biotechnol 27, 1177–1180.

Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. Trends Biotechnol 26, 126–131.

Deng, M.D., and Coleman, J.R. (1999). Ethanol synthesis by genetic engineering in cyanobacteria. Appl Environ Microbiol 65, 523–528.

Dexter, J., and Fu, P. (2009). Metabolic engineering of cyanobacteria for ethanol production. Energy Environ Sci 2, 857–864.

Fortman, J.L., Chhabra, S., Mukhopadhyay, A., Chou, H., Lee, T.S., Steen, E., and Keasling, J.D. (2008). Biofuel alternatives to ethanol: pumping the microbial well. Trends Biotechnol 26, 375–381.

González López, C.V., Acién Fernández, F.G., Fernández Sevilla, J. M., Sánchez Fernández, J.F., Cerón García, M.C., and Molina Grima, E. (2009). Utilization of the cyanobacteria *Anabaena* sp. ATCC 33047 in $CO_2$ removal processes. Bioresour Technol 100, 5904–5910.

Li, Y., Horsman, M., Wu, N., Lan, C.Q., and Dubois-Calero, N. (2008). Biofuels from microalgae. Biotechnol Prog 24, 815–820.

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.

 

Melis, A. (2009). Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci 177, 272–279.

Peterhansel, C., Niessen, M., and Kebeish, R.M. (2008). Metabolic engineering towards the enhancement of photosynthesis. Photochem Photobiol 84, 1317–1323.

Portis, A.R. Jr, and Parry, M.A. (2007). Discoveries in Rubisco (Ribulose 1,5-bisphosphate carboxylase/oxygenase): a historical perspective. Photosynth Res 94, 121–143.

Ramos, J.L., Duque, E., Gallegos, M.T., Godoy, P., Ramos-Gonzalez, M.I., Rojas, A., Teran, W., and Segura, A. (2002). Mechanisms of solvent tolerance in gram-negative bacteria. Annu Rev Microbiol 56, 743–768.

Rittmann, B.E. (2008). Opportunities for renewable bioenergy using microorganisms. Biotechnol Bioeng 100, 203–212.

Sheehan, J. (2009). Engineering direct conversion of $CO_2$ to biofuel. Nat Biotechnol 27, 1128–1129.

Spolaore, P., Joannis-Cassan, C., Duran, E., and Isambert, A. (2006). Commercial applications of microalgae. J Biosci Bioeng 101, 87–96.

Waltz, E. (2009). Biotech's green gold? Nat Biotechnol 27, 15–18.

Zhang, Y., Zhu, Y., Zhu, Y., and Li, Y. (2009). The importance of engineering physiological functionality into microbes. Trends Biotechnol 27, 664–672.

 