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Inhibition of Hydrogen Synthesis Increased Accumulations of Pigments in Cyanobacterium *Synechocystis* sp. PCC 6803

Nannaphat Sukkasam[1*] and Tanakarn Monshupanee[1*]

[1]*Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

**Corresponding author. E-mail: 6071955723@student.chula.ac.th, Tanakarn.M@chula.ac.th*
DOI:

ABSTRACT

Cyanobacterium *Synechocystis* sp. PCC 6803 produces one hydrogen gas molecule from two NADPH molecules via the only catalysis of the enzyme [NiFe]-hydrogenase composing of several subunits: HoxE, HoxF, HoxU, HoxY, and HoxH. Effects of the inhibition of hydrogen synthesis on the accumulation levels of other bioproducts have yet to be determined. Here, the two main catalytic subunits of [NiFe]-hydrogenase (HoxY and HoxH) were inactivated by the deletion of both *hoxY* and *hoxH* gene. Inactivation of both *hoxY* and *hoxH* gene did not have a deleterious effect to cell growth, suggesting that the hydrogen synthesis is not an essential process for cell viability. In addition, inactivation of both *hoxY* and *hoxH* genes significantly increased the accumulation levels of phycocyanin by 1.55 fold, and allophycocyanin by 1.49 fold. The biosynthesis of hydrogen (via hydrogenase), phycocyanin and allophycocyanin require the same substrate - NADPH. Thus, the inhibition of the hydrogen synthesis (via *hoxY* and *hoxH* inactivation) might help increase cellular NADPH level and mediate the enhanced production of phycocyanin and allophycocyanin.

Keywords: *Synechocystis* sp. PCC 6803, Cyanobacteria, Hydrogenase, Allophycocyanin, Phycocyanin

INTRODUCTION

Cyanobacteria are oxygenic photosynthesis prokaryotes. They assimilate solar energy, water, simple minerals, and carbon dioxide for their growth and produce various useful biomolecules (Ortega-Ramos et al., 2014), such as proteins, carbohydrates, pigments, hydrogen gas and bioplastic poly (3-hydroxybutyrate) (PHB) (Khanra et al., 2018; Mobin & Alam, 2017). Cyanobacterial oxygenic photosynthesis occurs by consuming sunlight as the sole energy and generate intracellular high-energy biomolecules: ATP and NADPH (Tschörtner, Lai, & Krömer, 2019). ATP and NADPH were utilized in cyanobacterial cellular metabolism including Calvin-Benson-Bassham cycle for carbon fixation, tricarboxylic acid (TCA) and other various biosynthesis pathways (Shimizu et al., 2015; Tang, Tang, & Blankenship, 2011). Of particular interest is the use of NADPH generated by ferredoxin-$NADP^+$ reductase in photosystem *I* for the biosynthesis of hydrogen, a number of pigments and bioplastic PHB (Khetkorn et al., 2017).

For hydrogen synthesis, two molecules of NADPH were catalyzed by hydrogenase which converts two protons and two electrons to one molecule of hydrogen gas (H_2) (Kruse & Hankamer, 2010; Sharma & Arya, 2017). For photosynthetic pigments, chlorophyll *a*, carotenoid, phycocyanin, allophycocyanin, and phycoerythrin which compose in photosystem *I* and photosystem *II* require NADPH as the co-factor for their biosynthesis (Dammeyer & Frankenberg-Dinkel, 2006; Ho, Soulier, Canniffe, Shen, & Bryant, 2017; Kramer & Evans, 2011; Mirkovic et al., 2017). Since the synthesis of hydrogen, the pigments require NADPH, inactivation of hydrogen synthesis might help increase cellular NADPH level and subsequently promote pigments production.

This study aims to increase cellular NADPH level in cyanobacteria by inactivating hydrogen synthesis and test whether this inactivated hydrogen synthesis can increase the production of the pigments in the well studied cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter, *Synechocystis*). In *Synechocystis*, [NiFe]-hydrogenase (ie. Hox, enzyme) is composed of several subunits, including HoxE, HoxF, HoxU, HoxY, and HoxH encoded by the gene *hoxE, hoxF, hoxU, hoxY*, and *hoxH*, respectively (Appel & Schulz, 1996; Schmitz et al., 2002). The main two catalytic subunits are HoxY and HoxH which reduce the protons form NADPH to generate H_2 (Vignais, Billoud, & Meyer, 2001). It has been showed that the knock-out *hoxY* and *hoxH* completely abolished hydrogenase activity in *Synechocystis* (Eckert et al., 2012). Thus this study performed the knock out of both *hoxY* and *hoxH* gene

to generate the Δ*hoxY-hoxH* mutant lacking hydrogen-gas synthesis. Then, the wild type and the Δ*hoxY-hoxH* mutant were compared for their cell growth, and the levels of pigments. Here we report the increased accumulation of pigments and PHB in Δ*hoxY-hoxH* mutant.

MATERIAL AND METHODS

Strains and culture condition. *Synechocystis* sp. PCC6803 (hereafter Synechocystis) was obtained from Pasture Culture collection. *Synechocystis* was cultured in BG11 liquid media (Rippka, Deruelles, Waterbury, Herdman, & Stanier, 1979) (300 mL) by adjusting the initial cell concentration to $OD_{730} = 0.5/mL$. Three culture conditions, namely the normal condition (NORMAL), the nitrogen deprivation (-N), and phosphorus deprivation (-P) (Monshupanee & Incharoensakdi, 2014). The cultures were supplied by atmospheric CO_2 concentration upon shaking at 160 rpm and cultured at 30 °C under continuous white light at 50 μmol/m^2/s. The cell biomass was then harvested on days 0, 1, 3, 7, 14, 21, and 28. *Escherichia coli* DH5α (Invitrogen, Massachusetts, USA) was used for plasmid production. *E. coli* was cultured in Luria–Bertani (LB) medium (Sambrook, 2001) at 37 °C upon shaking at 210 rpm.

Generation of ΔhoxY-hoxH mutant of *Synechocystis*. Plasmid for inactivating Δ*hoxY-hoxH* gene was constructed by commercial gene synthesis (GenScript, New Jersey, USA). The internal coding sequence of *hoxY-hoxH* operon (gene ID; sll1224 and sll1226) was deleted and inserted by kanamycin resistance gene (Kan^R) cassette (hereafter Δ*hoxY-hoxH:: *Kan^R). Kan^R cassette sequences were obtained from pUC4K (Taylor & Rose, 1988). Then the desired sequence Δ*hoxY-hoxH:: *Kan^R was cloned into plasmid pUC45. Next, pUC45 containing Δ*hoxY-hoxH:: *Kan^R was produced in *E. coli* DH5α and was transformed in to the wild type *Synechocystis* sp. PCC 6803 followed the method by Kufryk, Sachet, Schmetterer, & Vermaas, 2002. Briefly, the plasmid was incubated with *Synechocystis* cells for 6 hours to allow natural transformation to occur, and transformants were selected on BG11 agar medium containing 10 μg/mL kanamycin. The Δ*hoxY-hoxH:: *Kan^R transformant strain was segregated on 20 and 30 μg/mL kanamycin, respectively to ensure the presence of Δ*hoxY-hoxH:: *Kan^R genotype in all chromosomal copies of *Synechocystis*. The Δ*hoxY-hoxH:: *Kan^R genotype in the mutant was verified by gene specific primers (Primer XF: ATGAACCTGGTATTGTGC, Primer XR: CCTTCGTCTGGTTCTTAA,

Primer YF: TGCCTGTACTTCCTGTGG, and Primer YR: CCTTAAGACAGCCCAAAG) (Figure 1A).

Pigments analysis. Pigments extraction form *Synechocystis* cells was followed the method by Zavrel, Sinetova, & Červený, 2015. One mL of wet cell culture (1 OD_{730}/mL) were harvested and the wet biomass was added by 1 mL of cooling 99. 8% v/ v methanol, mixed, vortexed, and incubated for 30 minutes at 4 ºC in the dark. Then the supernatant was determined for the absorbance at 300- 800 nm ($A_{300} - A_{800}$). After that, pigments amounts were calculated following the equations of Bennett & Bogorad, 1973; Zavrel et al., 2015.

Chlorophyll *a* (Chl *a*; µg/mL) = $12.9447\ (A_{665} - A_{720})$

Carotenoids (Car; µg/mL) = $[1{,}000\ (A_{470} - A_{720}) - 2.86$ (Chl *a* (µg/mL))] / 221

Phycocyanin (PC; mg/mL) = $[A_{615} - (0.474 \times A_{652})] / 5.34$

Allophycocyanin (APC; mg/mL) = $[A_{652} - (0.208 \times A_{615})] / 5.09$

Phycoerythrin (PE; mg/mL) = $[A_{562} - (2.41 \times PC - 0.849 \times APC)] / 9.62$

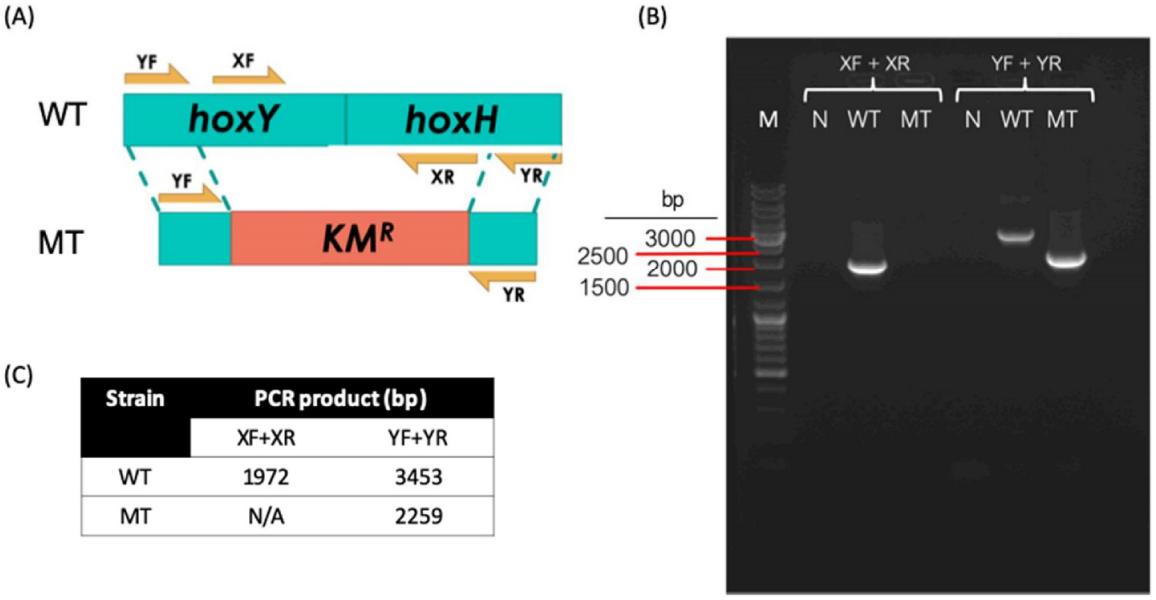
RESULTS

Inactivation of *hoxY* and *hoxH* gene.

The *hoxY* and *hoxH* contain 549-bp and 1,425-bp coding sequences, respectively. The 249-bp and 1,125-bp internal coding sequence of *hoxY* and *hoxH* gene were deleted and replaced by kanamycin resistant gene. The Δ*hoxY-hoxH* mutant strain can grow on BG11 agar containing kanamycin but the wild type cannot grow (not shown). PCR was used to verify the deletion of *hoxY* and *hoxH* in the Δ*hoxY-hoxH* mutant strain. By using the primers XF/XR (Figure 1A) that bind at the internal coding sequences of *hoxY* and *hoxH*, wild type showed the expected product at 1,972-bp while the *ΔhoxY-hoxH* mutant did not yield the product because the mutant lacks these internal coding sequences (Figure 1B and 1C). By using the primers YF/YR binding at the upstream and the downstream sequences of *hoxY* and *hoxH*, the wild type showed the 3,453-bp product as expected, and the Δ*hoxY-hoxH* mutant yielded the shorter 2,259-bp product due to the deletion of the internal sequences and the insertion of the kanamycin resistant gene (Figure 1B and 1C). Therefore, the inactivation of *hoxY* and *hoxH* was obtained in the Δ*hoxY-hoxH* mutant.

Cell density and biomass production.

Cell density was estimated using the optical density at 730 nm (OD_{730}). Wild type and the Δ*hoxY-hoxH* mutant have comparable OD_{730} under normal condition throughout the 28-day culture period (Figure 2). In addition, biomass productions of both strains were also at the comparable levels under normal growth condition (Figure 2). Thus, inactivation of *hoxY* and *hoxH* did not affect cell growth of *Synechocystis* under normal growth condition.



(C)

Strain	PCR product (bp)	
	XF+XR	YF+YR
WT	1972	3453
MT	N/A	2259

Figure 1. PCR verification of hoxY and hoxH inactivation. (A) Physical diagram of *hoxY* and *hoxH* genes in wild type (WT) and the Δ*hoxY-hoxH* mutant (MT). The figure is not drawn to scale. (B) Agarose gel electrophoresis of PCR products amplified from genomic DNA of WT and MT when use the two sets of primers: (XF+XR) and (YF+YR). Lane M is the DNA size marker; Lane N is the negative control (PCR without a DNA template). (C) The expected sizes of the PCR products obtained by the two sets of primers. N/A, not amplified.

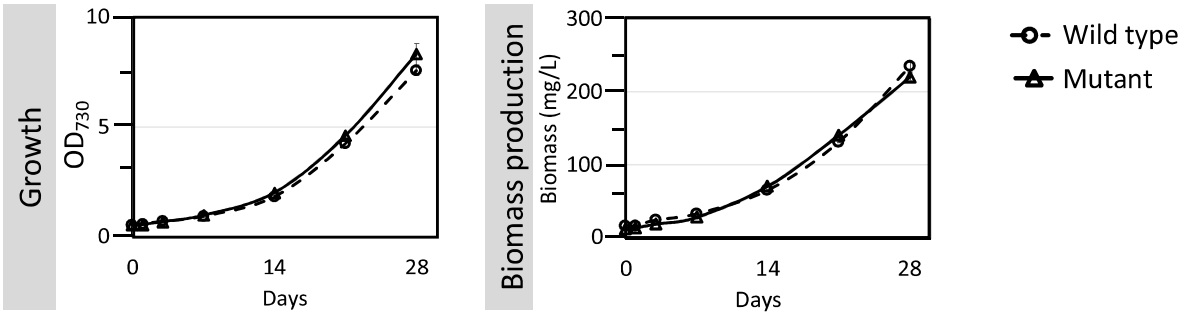


Figure 2. Cell growth and biomass production. The cell growth was measured by using OD_{730}. Data are shown as the mean ± SD derived from three independent cultures.

Effects of *hoxY-hoxH* deletion on pigment contents.

Pigment contents of the Δ*hoxY-hoxH* mutant were compared with those of wild type under normal growth condition, nitrogen deprivation (-N) and phosphorus deprivation (-P).

Chlorophyll *a* contents of the Δ*hoxY-hoxH* mutant were comparable to those of wild type under all three culture conditions. But with the only exception that under the normal growth condition at day 14, the chlorophyll *a* content of the mutant is 1.36 fold higher than that of wild type (Figure 3).

Carotenoid levels of the Δ*hoxY-hoxH* mutant are at the similar levels to those of wild type under all three culture conditions (Figure 3).

Interestingly, phycocyanin levels of the Δ*hoxY-hoxH* mutants were significantly higher than those of wild type by 1.46 fold under normal condition at day 21, and by 1.55 fold under –N condition at day 14. It should be noted that, under –N condition at day 28, there was no phycocyanin accumulation in the Δ*hoxY-hoxH* mutant, but wild type still accumulated phycocyanin. Thus, inactivation of *hoxY* and *hoxH* significantly affect phycocyanin accumulation under –N condition at the latter period (day 28) (Figure 2.). In contrats, under –P condition, phycocyanin levels are compareable in both strains (Figure 3).

Phycoerythrin levels of both strains are comparable under normal growth condition, -N condition and –P condition. There is the exception that under –N condition, the phycoerythrin level of the Δ*hoxY-hoxH* mutant was 3.11 fold higher than that of wild type but the statistical analysis indicates that these contents of wild type and the Δ*hoxY-hoxH* mutant are not significantly different (Figure 3).

Allophycocyanin contents of the Δ*hoxY-hoxH* mutant are not obviously different from those of wild type under -N condition and –P condition. But under normal growth condition, allophycocyanin contents of the Δ*hoxY-hoxH* mutant were significantly increased by 1.31 and 1.49 fold at 14 and 21 days, respectivly (Figure 3).

Overall, the Δ*hoxY-hoxH* mutant showed the significantly increased levels of phycocyanin by 1.55 fold and allophycocyanin by 1.49 fold, relative to those levels of wild type.

DISCUSSION

The *hoxY* and *hoxH* gene can be inactivated in *Synechocystis* without causing cell lethality. Thus HoxY and HoxH subunits of [NiFe]-hydrogenase are not essential for cell viability. Since HoxY and HoxH are vital catalytic subunits of [NiFe]-hydrogenase (Khanna & Lindblad, 2015), this data suggested that the loss of hydrogenase catalysis (i.e. loss of hydrogen gas production), did not cause deleterious effects to cell growth of *Synechocystis* (Figure 2). This data is consistent with the pervious report that the inactivation of all five subunits of [NiFe]-hydrogenase did not cause cell death in type *Synechocystis* (Eckert et al., 2012).

For pigments, inactivation of *hoxY* and *hoxH* did not obviously change the cellular levels of chlorophyll *a*, carotenoid, and phycoerythrin (Figure 3). Thus NADPH level may not the limiting factor for producing theses pigments, even though NADPH is required for their biosynthesis (Figure 4B.). In contrast, inactivation of *hoxY* and *hoxH* gene significantly increased the levels of phycocyanin and allophycocyanin under normal growth condition. We speculated that inactivation of *hoxY* and *hoxH* may result in the loss hydrogenate activity, hence might increase cellular NADPH level and help promote, phycocyanin and allophycocyanin synthesis (Figure 4). This result was the first report of the effect of inactivation of hydrogen synthesis on the levels of these two pigments.

In previous report, increased levels of chlorophyll *a* was obtained by the inactivation of the porphyrin synthetic pathway that use the same substrate with chlorophyll *a* synthesis (Sobotka, Tichy, Wilde, & Hunter, 2011). In addition, the levels of the pigments (coproporphyrin III, protoporphyrin IX, Mg-protoporphyrin IX, Mg-protoporphyrin IX and chlorophyll *a*) were increased after exposing cyanobacterial cells to N-methyl mesoporphyrin IX, the metabolic inhibitor that blocks the pathways that consume the same

substrates to those of the pigment biosynthesis (Pazderník, Mareš, Pilný, & Sobotka, 2019).

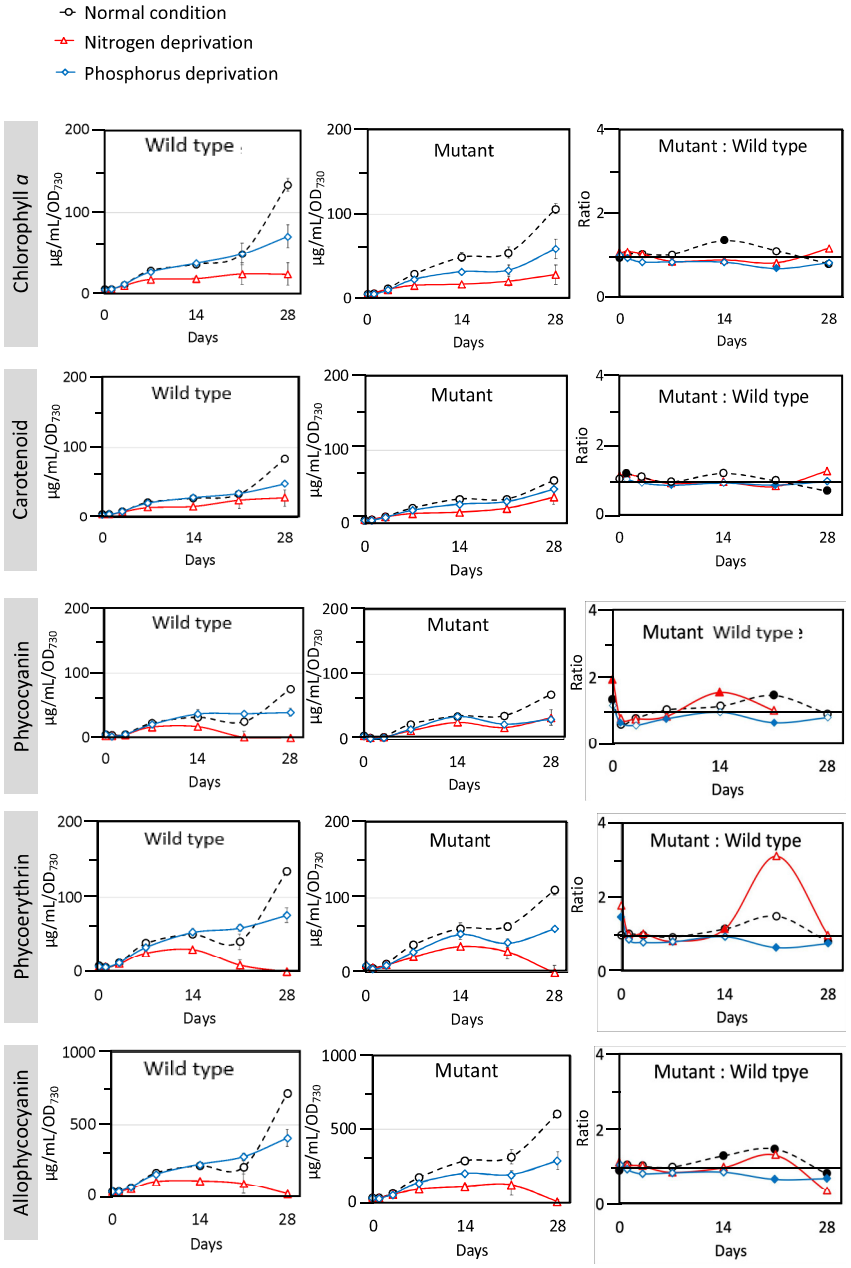


Figure 3. Pigment contents. The data shows the mean ± SD derived from three independent cultures. The left panels are the ratios of the contents between: the Δ*hoxY-hoxH* mutant: wild type. The filled dots mark significantly different values (Student's t-test, $p \leq 0.0$), and the empty dots represent statistically similar values (Student's t-test, $p > 0.05$).

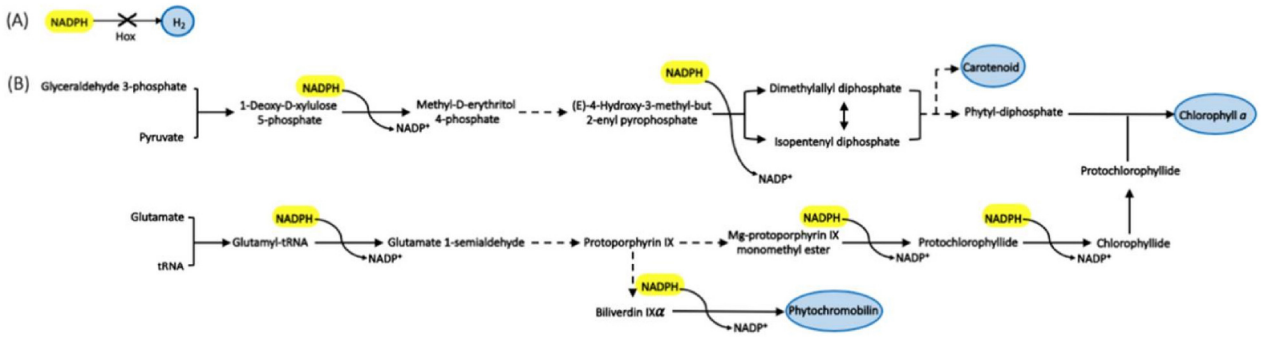


Figure 4. Biosynthetic pathway of hydrogen gas, pigments that require NADPH as co-factor for their biosynthesis in *Synechocystis* sp. PCC 6803. The inactivation of Hox enzyme for hydrogen production was indicated by cross line. NADPH was high-lighted by yellow and the bioproducts were indicated by blue circle. Direct reactions (continuous-line arrows) and abbreviated pathways (broken-line arrows) are indicated. The diagram was redrawn from Bautista et al., 2005; Brzezowski, Richter, & Grimm, 2015; Fujita, Tsujimoto, & Aoki, 2015; Hondo et al., 2015; Koch, Doello, Gutekunst, & Forchhammer, 2019;Maarleveld, Boele, Bruggeman, & Teusink, 2014; Masuda &Fujita, 2008; Salinas & Grimm, 2012; Stanier & Cohen-Bazire, 1977; Takaichi, Maoka, & Masamoto, 2001; Tanaka & Tanaka, 2006; Terry & Kendrick, 1999; Willows et al., 2000; Zhang, Selão, Selstam, & Norling, 2015.

CONCLUSION

The two main catalytic subunits of [NiFe]-hydrogenase (HoxY and HoxH) were inactivated by deleting *hoxY* and *hoxH* gene in *Synechocystis*. Thus, the hydrogen production is not essential for *Synechocystis* cell viability under normal growth condition. Deleting *hoxY* and *hoxH* significantly increased the level of phycocyanin and allophycocyanin. Since the inactivated [NiFe]-hydrogenase (via deleting *hoxY* and *hoxH* gene) would abolish NADPH consumption for hydrogen production; thus the inactivation of [NiFe]-hydrogenase may increase allophycocyanin accumulation via the increased NADPH levels of the cells.

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REFERENCES

Appel, J., & Schulz, R. (1996). Sequence analysis of an operon of a NAD(P)-reducing nickel hydrogenase from the cyanobacterium *Synechocystis* sp. PCC 6803 gives additional evidence for direct coupling of the enzyme to NAD(P)H-dehydrogenase (complex 1). *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, 1298(2), 141-147.

Bautista, J. A., Tracewell, C. A., Schlodder, E., Cunningham, F. X., Jr., Brudvig, G. W., & Diner, B. A. (2005). Construction and characterization of genetically modified *Synechocystis* sp. PCC 6803 photosystem *II* core complexes containing carotenoids with shorter pi-conjugation than beta-carotene. *The Journal of Biological Chemistry*, 280(46), 38839-38850.

Bennett, A., & Bogorad, L. (1973). Complementary chromatic adaptation in a filamentous blue-green alga. *The Journal of Cell Biology*, 58(2), 419.

Brzezowski, P., Richter, A. S., & Grimm, B. (2015). Regulation and function of tetrapyrrole biosynthesis in plants and algae. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1847(9), 968-985.

Carstensen, A., Herdean, A., Schmidt, S. B., Sharma, A., Spetea, C., Pribil, M., & Husted, S. (2018). The Impacts of Phosphorus Deficiency on the Photosynthetic Electron Transport Chain. *Plant Physiology*, 177(1), 271.

Dammeyer, T., & Frankenberg-Dinkel, N. (2006). Insights into Phycoerythrobilin Biosynthesis Point toward Metabolic Channeling. *Journal of Biological Chemistry*, 281(37), 27081-27089.

Eckert, C., Boehm, M., Carrieri, D., Yu, J., Dubini, A., Nixon, P. J., & Maness, P. C. (2012). Genetic analysis of the Hox hydrogenase in the cyanobacterium *Synechocystis* sp. PCC 6803 reveals subunit roles in association, assembly, maturation, and function. *The Journal of Biological Chemistry*, 287(52), 43502-43515.

Fujita, Y., Tsujimoto, R., & Aoki, R. (2015). Evolutionary aspects and regulation of tetrapyrrole biosynthesis in cyanobacteria under aerobic and anaerobic environments. *Life*, 5(2).

Ho, M.-Y., Soulier, N. T., Canniffe, D. P., Shen, G., & Bryant, D. A. (2017). Light regulation of pigment and photosystem biosynthesis in cyanobacteria. *Current Opinion in Plant Biology*, 37, 24-33.

Khanna, N., & Lindblad, P. (2015). Cyanobacterial hydrogenases and hydrogen metabolism revisited: recent progress and future prospects. *International Journal of Molecular Sciences*, 16(5), 10537-10561.

Khanra, S., Mondal, M., Halder, G., Tiwari, O. N., Gayen, K., & Bhowmick, T. K. (2018). Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: A review. *Food and Bioproducts Processing*, 110, 60-84.

Khetkorn, W., Rastogi, R. P., Incharoensakdi, A., Lindblad, P., Madamwar, D., Pandey, A., & Larroche, C. (2017). Microalgal hydrogen production – A review. *Bioresource Technology*, 243, 1194-1206.

Koch, M., Doello, S., Gutekunst, K., & Forchhammer, K. (2019). PHB is produced from glycogen turn-over during nitrogen starvation in *Synechocystis* sp. PCC 6803. *International Journal of Molecular Sciences,* 20(8).

Kramer, D. M., & Evans, J. R. (2011). The importance of energy balance in improving photosynthetic productivity. *Plant Physiology*, 155(1), 70-78.

Kruse, O., & Hankamer, B. (2010). Microalgal hydrogen production. *Current Opinion in Biotechnology*, 21(3), 238-243.

Kufryk, G. I., Sachet, M., Schmetterer, G., & Vermaas, W. F. J. (2002). Transformation of the cyanobacterium *Synechocystis* sp. PCC 6803 as a tool for genetic mapping: optimization of efficiency. *FEMS Microbiology Letters*, 206(2), 215-219.

Maarleveld, T. R., Boele, J., Bruggeman, F. J., & Teusink, B. (2014). A data integration and visualization resource for the metabolic network of *Synechocystis* sp. PCC 6803. *Plant Physiology*, 164(3), 1111.

Masuda, T., & Fujita, Y. (2008). Regulation and evolution of chlorophyll metabolism. *Photochemical and Photobiological Sciences*, 7(10), 1131-1149.

Mirkovic, T., Ostroumov, E. E., Anna, J. M., van Grondelle, R., Govindjee, & Scholes, G. D. (2017). Light absorption and energy transfer in the antenna complexes of photosynthetic organisms. *Chemical Reviews*, 117(2), 249-293.

Mobin, S., & Alam, F. (2017). Some promising microalgal species for commercial applications: A review. *Energy Procedia*, 110, 510-517.

Monshupanee, T., & Incharoensakdi, A. (2014). Enhanced accumulation of glycogen, lipids and polyhydroxybutyrate under optimal nutrients and light intensities in the cyanobacterium *Synechocystis* sp. PCC 6803. *Journal of Applied Microbiology*, 116(4), 830-838.

Ortega-Ramos, M., Jittawuttipoka, T., Saenkham, P., Czarnecka-Kwasiborski, A., Bottin, H., Cassier-Chauvat, C., & Chauvat, F. (2014). Engineering *Synechocystis* PCC 6803 for hydrogen production: influence on the tolerance to oxidative and sugar stresses. *PLOS ONE*, 9(2), e89372.

Pazderník, M., Mareš, J., Pilný, J., & Sobotka, R. (2019). The antenna-like domain of the cyanobacterial ferrochelatase can bind chlorophyll and carotenoids in an energy-dissipative configuration. *Journal of Biological Chemistry*, 294(29), 11131-11143.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology*, 111(1), 1-61.

Salinas, A., & Grimm, B. (2012). The magnesium branch of tetrapyrrole biosynthesis: from magnesium protoporphyrin IX to chlorophyll a/b. In *Handbook of Porphyrin Science* (Volume 28), pp. 85-115: World Scientific Publishing Company.

Sambrook, J. (2001). *Molecular cloning : a laboratory manual*: Third edition. Cold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory Press, [2001] ©2001.

Schmitz, O., Boison, G., Salzmann, H., Bothe, H., Schütz, K., Wang, S.-h., & Happe, T. (2002). HoxE—a subunit specific for the pentameric bidirectional hydrogenase complex (HoxEFUYH) of cyanobacteria. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1554(1), 66-74.

Sharma, A., & Arya, S. K. (2017). Hydrogen from algal biomass: A review of production process. *Biotechnology Reports*, 15, 63-69.

Shimizu, R., Dempo, Y., Nakayama, Y., Nakamura, S., Bamba, T., Fukusaki, E., & Fukui, T. (2015). New insight into the role of the calvin cycle: Reutilization of CO_2 emitted through sugar degradation. *Scientific Reports*, 5, 11617-11617.

Sobotka, R., Tichy, M., Wilde, A., & Hunter, C. N. (2011). Functional assignments for the carboxyl-terminal domains of the ferrochelatase from *Synechocystis* PCC 6803: the CAB domain plays a regulatory

role, and region II is essential for catalysis. *Plant Physiology*, 155(4), 735-1747.

Stanier, R. Y., & Cohen-Bazire, G. (1977). Phototrophic prokaryotes: the cyanobacteria. *Annual Review of Microbiology*, 31, 225-274.

Takaichi, S., Maoka, T., & Masamoto, K. (2001). Myxoxanthophyll in *Synechocystis* sp. PCC 6803 is myxol 2'-dimethyl-fucoside, (3R,2'S)-myxol 2'-(2,4-di-O-methyl-alpha-L-fucoside), not rhamnoside. Plant and Cell Physiology, 42(7), 756-762.

Tanaka, A., & Tanaka, R. (2006). Chlorophyll metabolism. *Current Opinion in Plant Biology*, 9(3), 248-255

Tang, K.-H., Tang, Y., & Blankenship, R. (2011). Carbon metabolic pathways in phototrophic bacteria and their broader evolutionary implications. *Frontiers in Microbiology*, 2, 165.

Taylor, L. A., & Rose, R. E. (1988). A correction in the nucleotide sequence of the Tn903 kanamycin resistance determinant in pUC4K. *Nucleic Acids Research*, 16(1), 358-358.

Terry, M. J., & Kendrick, R. E. (1999). Feedback inhibition of chlorophyll synthesis in the phytochrome chromophore-deficient aurea and yellow-green-2 mutants of tomato. *Plant physiology*, 119(1), 143-152.

Tschörtner, J., Lai, B., & Krömer, J. O. (2019). Biophotovoltaics: Green power generation from sunlight and water. *Frontiers in Microbiology*, 10, 866.

Vignais, P. M., Billoud, B., & Meyer, J. (2001). Classification and phylogeny of hydrogenases1. *FEMS Microbiology Reviews*, 25(4), 455-501.

Willows, R. D., Mayer, S. M., Foulk, M. S., DeLong, A., Hanson, K., Chory, J., & Beale, S. I. (2000). Phytobilin biosynthesis: the *Synechocystis* sp. PCC 6803 heme oxygenase-encoding ho1 gene complements a phytochrome-deficient *Arabidopsis thalianna* hy1 mutant. *Plant Molecular Biology,* 43(1), 113-120.

Zavrel, T., Sinetova, M. A., & Červený, J. (2015). Measurement of chlorophyll *a* and carotenoids concentration in cyanobacteria. *Bio-protocol*, 5(9), e1467.

Zhang, L., Selão, T. T., Selstam, E., & Norling, B. (2015). Subcellular localization of carotenoid biosynthesis in *Synechocystis* sp. PCC 6803. PLOS One, 10(6), e0130904.