Mitigating guanidine toxicity and manipulating circadian clock for enhanced ethylene production in engineered cyanobacteria

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We are developing cyanobacteria as optimized cell factories for producing biofuels and other renewable biochemicals. Our *long-term goal* is to develop technologies that can be used to optimize cyanobacteria and other microbes for producing renewable chemicals at commercially feasible rates and yields. One objective of our project is to achieve sustained production of ethylene in the engineered *efe*-expressing cyanobacterium *Synechococcus elongatus* through removal of the toxic byproduct guanidine. A second objective is to manipulate the biological clock in the engineered ethylene-producing and guanidine-degrading strains to further enhance and stabilize photosynthetic production of ethylene.

Due to biotechnological interests in developing an alternative pathway for renewable production of ethylene, which is the most highly produced organic compound in the petro-chemical industry, the *efe* gene (encoding the ethylene-forming enzyme; EFE) from *Pseudomonas syringae* (a plant pathogen) has been introduced into a variety of microbial species[1]. Some hosts, *e.g.*, *Pseudomonas putida* KT2440 and the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803), have been able to accommodate stable, high-level expression of EFE and thereby sustain enhanced production of ethylene[2]. Other species, such as cyanobacterium *Synechococcus elongatus* PCC 7942 (hereafter *Synechococcus* 7942), however, have not been able to tolerate high-level expression of EFE, and the recombinant strains suffered severe growth inhibition[3, 4] that was rescued by spontaneous chromosomal mutations that abolished the expression of functional EFE and therefore ethylene formation[3, 4].

In this study, we show that accumulation of guanidine (a byproduct of biological ethylene production) significantly inhibits the growth of cyanobacterial cells, and destabilizes their genome in response to recombinant EFE expression. We found that Sll1077, previously annotated as an agmatinase in the arginase superfamily in *Synechocystis* 6803[5, 6], is more likely a "guanidinase", because it degrades guanidine rather than agmatine to urea. This result is consistent with the finding that there is a conserved sequence motif of the guanidine riboswitch upstream of the *sll1077* ORF. *Synechococcus* 7942 lacks a homologous enzyme in its genome and is unable to mitigate guanidine toxicity. Heterologous expression of Sll1077 in a recombinant *Synechococcus* 7942 strain confers the ability to degrade guanidine into non-toxic urea. Co-expression of Sll1077 and EFE in *Synechococcus* 7942 stabilizes the genome of the resultant strain and leads to sustained production of ethylene from light and CO_2.

Next, we sought to further enhance the bioethylene production through manipulating the biological clock. The cyanobacterial circadian clock exerts control over global gene regulation and oscillations of intracellular metabolism. Our previous studies have proven that overexpression of a key clock positive regulator could constantly enhance expression of both endogenous and exogenous genes in *Synechococcus* 7942[7]. We recently found that mutations of clock negative regulators and deletion of some clock output pathway players could even further enhance expression of genes of interest. Our preliminary results showed that ethylene production in the engineered *Synechococcus* 7942 strain is under regulation of the circadian clock. Based on these findings, we have generated a series of engineered *Synechococcus* 7942 strains, and further characterization is underway. If successful, manipulation of the circadian clock genes could be a promising approach for enhancing production of biofuels and chemicals in cyanobacteria.

References

1. C. Eckert, W. Xu, W. Xiong, S. Lynch, J. Ungerer, L. Tao, R. Gill, P. C. Maness and J. Yu, *Biotechnol Biofuels*, 2014, **7**, 33.
2. B. Wang, C. Eckert, P. C. Maness and J. Yu, *ACS Synth Biol*, 2018, **7**, 276-286.
3. M. Sakai, Ogawa, T., Matsuoka, M., Fukuda, H., *Journal of Fermentation and Bioengineering*, 1997, **84**, 434-443.
4. K. Takahama, M. Matsuoka, K. Nagahama and T. Ogawa, *J Biosci Bioeng*, 2003, **95**, 302-305.
5. A. Sekowska, A. Danchin and J. L. Risler, *Microbiology (Reading)*, 2000, **146 (Pt 8)**, 1815-1828.
6. J. W. Nelson, R. M. Atilho, M. E. Sherlock, R. B. Stockbridge and R. R. Breaker, *Mol. Cell*, 2017, **65**, 220-230.
7. Y. Xu, P. D. Weyman, M. Umetani, J. Xiong, X. Qin, Q. Xu, H. Iwasaki and C. H. Johnson, *Current biology : CB*, 2013, **23**, 2365-2374.

This work was supported by Department of Energy award DE-SC0019404.