Metabolic engineering of cyanobacteria for enhanced production of ethylene and free fatty acids

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We are developing cyanobacteria as optimized cell factories for producing biofuels and other renewable biochemicals. One approach takes advantage of the global control of gene expression by the circadian "biological clock" to enhance photosynthetic production of ethylene and free fatty acids (FFAs). The second approach involves stabilizing expression of the ethylene forming enzyme (EFE) through removal of the inhibitory byproduct guanidine. The third approach involves metabolic engineering to circumvent native regulation of fatty acid biosynthesis.

In the first approach, we are investigating the ability of circadian clock reprogramming[1] to enhance product formation in three different species or strains of cyanobacteria: *Synechococcus* sp. PCC 7942 and PCC 7002, and *Synechocystis* sp. PCC 6803. We are using both PCC 6803 and PCC 7942 to produce the economically important chemical feedstock ethylene. Ethylene can be produced from CO_2 in PCC 6803 by overexpressing a single ethylene-forming enzyme (EFE) that derives from a plant pathogen.[2] Preliminary studies showed that ethylene production in this engineered *Synechocystis* is under regulation of the circadian clock, but attempts to manipulate the clock in PCC 6803 have been challenging due to the complexity and redundancy of circadian clock regulation in this species.

Ethylene can also be produced by expressing EFE in PCC 7942[3], which has a well characterized circadian clock, but the transgenic strains exhibited instability which is at least partially due to the toxicity of guanidine that is generated concomitantly with ethylene from the EFE reaction[4]. Therefore, we overexpressed a guanidine-degrading (GD) enzyme from PCC 6803 in PCC 7942, after which the cells showed much higher tolerance to exogenous guanidine as well as to the expression of EFE. Recently we have implemented two inducible systems to regulate the expression of EFE in PCC 7942, in order to further enhance strain stability. These stabilized strains of PCC 7942 will enable circadian clock reprogramming to be tested in a more predictable ethylene-producing host species.

Finally, we are developing strains of cyanobacteria that are optimized for production of FFAs, which are precursors to diesel fuels and other high-value oleochemicals including fatty alcohols, methyl ketones, and olefins[5]. We are engineering PCC 7002 to produce medium chain FFAs by overexpressing thioesterases that target the corresponding acyl-ACP intermediates made via fatty acid biosynthesis. Genes are integrated at the site of key acyl-ACP synthetases, thereby eliminating futile cycles. Early strains are producing titers comparable to the best presented in the literature, albeit in shorter production times given the faster growth of PCC 7002. Ongoing work is examining other rate limiting steps in fatty acid biosynthesis as well as product export and toxicity.

Unlike PCC 6803 and PCC 7942, it has not been reported whether a circadian system

exists in PCC 7002. To test if PCC 7002 shows circadian rhythmicity, we have also generated two kinds of luminescence reporters using *Vibrio harveyi* luciferase encoded by *luxA* and *luxB* (*luxAB*) genes. Both reporter constructs exhibited robust circadian rhythms in PCC 7002 under constant light conditions. Moreover, the circadian rhythms of PCC 7002 showed longer circadian periods and different phase angles at 30°C relative to those in PCC 7942. The PCC 7002 rhythms also displayed two other diagnostic circadian properties; they were well entrained by light/dark cycles and compensated over a broad range of temperatures. Therefore, we have discovered that a potent circadian clock exists in PCC 7002, and our recent data have demonstrated that reprogramming of the PCC7002 circadian clock can also promote expression levels of foreign reporter genes. In future studies we will attempt to manipulate it to further enhance production of FFA and other renewable biochemicals.

References

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