

SUSTAINABLE PRODUCTION OF AROMATIC AMINO ACIDS BY ENGINEERED CYANOBACTERIA

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ABSTRACT

With the increasing concern of climate change, engineering strategies to capture and fix carbon dioxide to produce valuable chemicals is a promising proposition. Metabolic engineering efforts have recently been focused on using cyanobacteria as hosts for the production of biochemicals due to their ability to utilize carbon dioxide and sunlight as the sole carbon and energy sources, respectively. Unlike fermentation which uses plant derived sugars, cyanobacterial biochemical production does not compete for arable land that can be utilized for food production. Aromatic amino acids such as L-phenylalanine (Phe) and L-tryptophan (Trp) are essential amino acids since they cannot be synthesized by animals and thus needed as supplements. They are valuable as animal feed supplements in the agricultural industry and find wide applications in the food, cosmetic and pharmaceutical industries as precursors. However, investigation of cyanobacteria for production of aromatic amino acids such as Phe and Trp is limited. This dissertation studies (i) combining random mutagenesis and metabolic engineering techniques for Trp and Phe production in *Synechocystis* sp. PCC 6803, (ii) development of a fast-growing cyanobacteria strain *Synechococcus elongatus* PCC 11801 for Phe production and (iii) investigating the effect of creation of Phe sink on photosynthetic efficiency under different light intensities.

Aromatic amino acid biosynthesis is tightly regulated by feedback inhibition in cyanobacteria. To enable overproduction of Trp in *Synechocystis* sp PCC 6803, we utilized chemical mutagenesis coupled with analog selection followed by genome sequencing to identify single nucleotide polymorphisms (SNPs) responsible for the Trp overproduction phenotypes. Interestingly, overproducers had mutations in the competing Phe biosynthetic pathway gene chorismate mutase (CM) which resulted in a lower enzyme activity and redirection of flux to Trp. We subsequently overexpressed genes encoding feedback resistant enzymes in our randomly engineered Trp overproducing strain. The best strain isolated was able to accumulate 212 ± 23 mg/L Trp in 10 days under 3% (vol/vol) CO₂. We demonstrate that combining random mutagenesis and metabolic engineering is superior to either approach alone.

Initial efforts in engineering cyanobacteria have resulted in low titers and productivities due to slow growth. Recently a fast-growing cyanobacterial strain *Synechococcus elongatus* PCC

11801 was discovered with growth rates comparable to yeast. Due to the lack of well characterized synthetic biology tools available for metabolic engineering of this strain, we use two rounds of ultraviolet (UV) mutagenesis and analog selection to develop Phe overproducing strains. The best strain obtained using this strategy can produce 1.2 ± 0.1 g/L of Phe in 3 days under 3% (vol/vol) CO₂. This is the highest titer and productivity for Phe production currently reported by cyanobacteria highlighting the promise of engineering fast-growing strains for biochemical production.

Interestingly, Phe overproduction does not compete with growth but happens by fixing carbon at a higher rate. It is thought that the introduction of this carbon and energy sink relieves “sink limitation” by improving light use. However, neither the molecular mechanism nor the effect of light on enhancement in carbon fixation by introduction of an additional sink are known. Therefore, we investigated the effect of light intensity on photosynthetic efficiency, linear and cyclic electron flow in the strain containing the Phe sink. Our results indicate that under excess light, introduction of the Phe sink improves carbon fixation by improving photosynthetic efficiency and substantially reducing the cyclic electron flow around photosystem I (PSI). Taken together, our results show the previously untapped potential of cyanobacteria to improve carbon fixation by the unintuitive strategy of introducing a native carbon product sink and highlight the importance of the light environment on its performance.

Although further improvements in titer, productivity, and scale up will be necessary for cyanobacteria to compete economically at the industrial scale, this dissertation adds to the scientific knowledge and techniques for further metabolic engineering efforts.