Chemical Engineering Doctoral Defense

ENGINEERING SYNECHOCOCCUS SP. UTEX 2973 AND DEVISING CARBON DIOXIDE UPTAKE STRATEGIES FOR AMINO ACID AND BIOPLASTIC PRODUCTION

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Abstract

Amino acids and related targets are typically produced by well-characterized heterotrophs including Corynebacterium glutamicum and Escherichia coli. Recent efforts have sought to supplant these sugar-intensive processes through the metabolic engineering of cyanobacteria, which instead can directly utilize atmospheric CO2 and sunlight. One of the most promising among recently discovered photoautotrophic strains is Synechococcus elongatus UTEX 2973 (hereafter UTEX 2973), which has been reported to have doubling times as low as 1.9 hours. While encouraging, there are still major challenges preventing the widespread industrial uptake of recombineered cyanobacteria, chief among them the scarcity of genetic tools and parts with which to engineer production strains. Here, UTEX 2973 was engineered to overproduce L-lysine through the heterologous expression of feedback-resistant copies of lysC and ybjE from Escherichia coli, aided by the characterization of novel combinations of genetic parts and expression sites. At maximum, a plasmid-based expression system attained a titer of 556 ± 62.3 mg/L L-lysine after 120 hours, surpassing a prior report of photoautotrophic L-lysine bioproduction. Modular extension of the pathway led to novel photsynthetic production of the corresponding diamine cadaverine (55.3 ± 6.7 mg/L at 96 hours) and dicarboxylate glutarate (67.5 ± 2.2 mg/L at 96 hours). Lastly, mass transfer experiments were carried out to determine the favorable CO2 loading of BG-11 media supplemented with various amines, including cadaverine. Cyanobacteria grown in the presence of a subset of these amines grew worse than a no-amine control, demonstrating the need for additional tolerance engineering to successfully implement this strategy.

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