5-aminolevulinic Acid Production Pathway Introduced into *Escherichia coli*

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Escherichia coli W3110 strain was metabolically engineered to produce 5-aminolevulinic acid (ALA). First, Rhodobacter sphaeroides hemA gene was codon-optimized and cloned into high copy number plasmid pKE112. Second, plasmid pKE112hemA was introduced into the lacI-deleted WL3110 strain; the WL3110 (pKE112hemA) strain produced 0.249 g/L of ALA. Third, in silico knock-out simulation was carried out to identify additional gene knock-out targets to further improve ALA production. The gcvTHP genes (glycine cleavage system) were predicted as knockout targets. The JW01 strain (WL3110 ΔgcvTHP pKE112hemA) produced 1.17g/L of ALA, which was 4.7 times higher than that obtained with the base strain. Finally, in order to increase the succinyl-CoA pool, the glyoxylate shunt flux was enhanced by the deletion of the iclR and sdhAB genes, while the TCA cycle flux was reinforced by the deletion of ptsG gene. The JW03 strain (JW01 ΔiclR ΔsdhAB ΔptsG pKE112hemA) was able to produce 1.72g/L of ALA. Fed-batch culture of the JW03 (pKE112hemA) strain resulted in the production of 5.77 g/L of ALA in 41 h.
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