Metabolic Engineering of Nonmodel Yeast *Issatchenkia orientalis* SD108 for 5-Aminolevulinic Acid Production

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Background/Objective

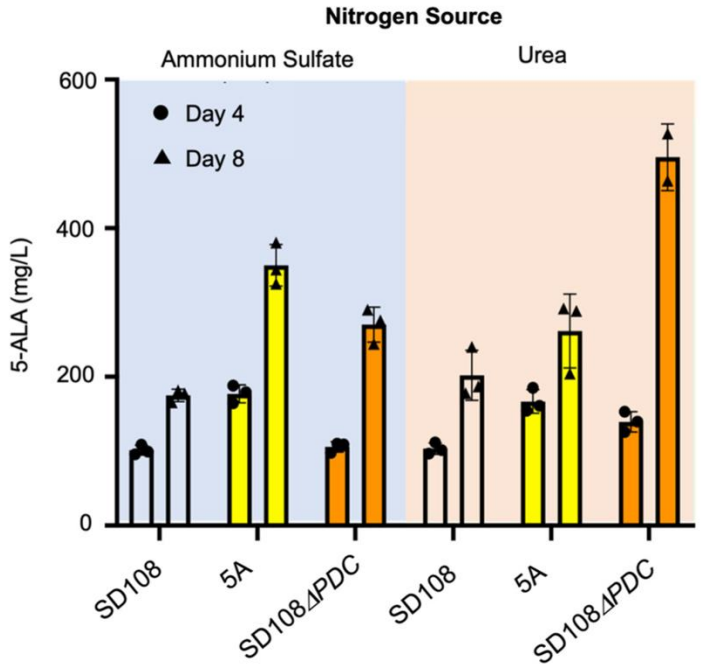
5-aminolevulinic acid (5-ALA) is a nonproteinogenic amino acid with many applications in the pharmaceutical and agricultural industries that can be produced by biosynthesis. However, 5-ALA is not stable in neutral conditions, which is required for model organisms to grow. We engineered an acid-tolerant yeast, *Issatchenkia orientalis* SD108, to address this limitation.

Approach

We first tested *I. orientalis* tolerance to 5-ALA and fitness as a production host. We then enhanced production by choosing an optimal ALA synthetase (ALAS) gene, optimizing plasmid design, overexpressing a transporter, and increasing gene copy number. Furthermore, we redirected the carbon flux to 5-ALA production using the pyruvate decarboxylase (PDC) knockout strain (SD108ΔPDC). Finally, we tested different nitrogen sources to increase the production in a minimal medium.

Results

We first discovered that the cell growth rate of *I. orientalis* SD108 was boosted by 5-ALA, and its endogenous ALAS gene showed higher activity than homologs from other yeasts. The 5-ALA titer was improved from 28 mg/L to 120-, 150-, and 300 mg/L, after we optimized the plasmid design, transporter, and gene copy number, respectively. Metabolic flux enhancement and culturing with urea increased the titer to 510 mg/L, a 13-fold enhancement.



Effect of nitrogen source on 5-ALA production in SD108, 5A, and SD108ΔPDC strains.

Significance/Impacts

This study demonstrates the acid-tolerant *I. orientalis* SD108ΔPDC has a high potential for 5-ALA production at a large scale in the future.

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