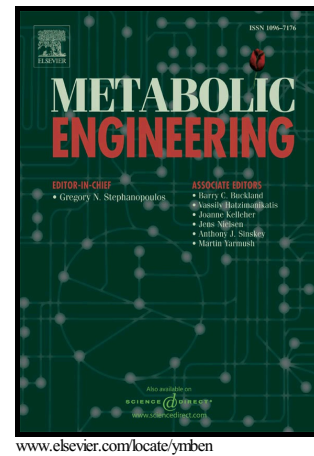


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**Photosynthesis-dependent biosynthesis of medium chain-length fatty acids and alcohols**

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**Abstract**

Cyanobacteria can directly channel atmospheric CO<sub>2</sub> into a wide range of versatile carbon products such as fatty acids and fatty alcohols with applications including fuel, cosmetics, and health products. Works on alcohol production in cyanobacteria have so far focused on either long (C12-C18) or short (C2-C4) chain-length products. In the present work, we report the first synthetic pathway for 1-octanol (C8) biosynthesis in *Synechocystis* sp. PCC 6803, employing a carboxylic acid reductase and C8-preferring fatty acyl-ACP thioesterase. The first engineered strain produced 1-octanol but exhibited poor productivity and cellular health issues. We therefore proceeded to systematically optimize the strain and cultivation conditions in order to understand what the limiting factors were. The identification of optimal promoters and ribosomal binding sites, in combination with isopropyl myristate solvent overlay, resulted in a combined (C8-OH and C10-OH) titer of more than 100 mg/L (a 25-fold improvement relative to the first engineered strain) and a restoration of cellular health. Additionally, more than 905 mg/L 1-octanol was produced when the strain expressing *sfp* (phosphopantetheinyl transferase) and *car* (carboxylic acid reductase) was fed with octanoic acid. A combination of feeding experiments and protein quantification indicated that the supply of octanoic acid from the introduced thioesterase, and possibly also native fatty acid synthesis pathway, were the main bottlenecks of the pathway.

**Keywords:** 1-octanol, biofuel, octanoic acid, carboxylic acid reductase, synthetic metabolic pathway, cyanobacteria, genetic instability

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