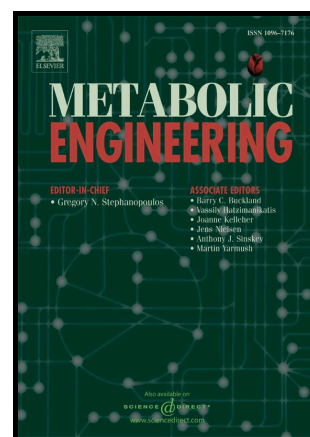


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PII: S1096-7176(17)30451-2
DOI: <https://doi.org/10.1016/j.ymben.2018.02.014>
Reference: YMBEN1353

To appear in: *Metabolic Engineering*

Received date: 6 December 2017
Revised date: 12 January 2018
Accepted date: 25 February 2018

Cite this article as: Rui Miao, Hao Xie, Felix Ho and Peter Lindblad, Protein engineering of α -ketoisovalerate decarboxylase for improved isobutanol production in *Synechocystis* PCC 6803, *Metabolic Engineering*, <https://doi.org/10.1016/j.ymben.2018.02.014>

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Protein engineering of α -ketoisovalerate decarboxylase for improved isobutanol production in *Synechocystis* PCC 6803

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Abstract

Protein engineering is a powerful tool to modify e.g. protein stability, activity and substrate selectivity. Heterologous expression of the enzyme α -ketoisovalerate decarboxylase (Kivd) in the unicellular cyanobacterium *Synechocystis* PCC 6803 results in cells producing isobutanol and 3-methyl-1-butanol, with Kivd identified as a potential bottleneck. In the present study, we used protein engineering of Kivd to improve isobutanol production in *Synechocystis* PCC 6803. Isobutanol is a flammable compound that can be used as a biofuel due to its high energy density and suitable physical and chemical properties. Single replacement, either Val461 to isoleucine or Ser286 to threonine, increased the Kivd activity significantly, both *in vivo* and *in vitro* resulting in increased overall production while isobutanol production was increased more than 3-methyl-1-butanol production. Moreover, among all the engineered strains examined, the strain with the combined modification V461I/S286T showed the highest (2.4

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