MODEL-GUIDED METABOLIC ENGINEERING OF *PSEUDOMONAS TAIWANENSIS* VLB120 FOR THE PRODUCTION OF METHYL KETONES

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Aliphatic methyl ketones are discussed as promising novel diesel blendstocks because of their favorable cetane numbers. To achieve sustainable production of these compounds, bio-based production in engineered microbes is followed and successful synthesis in *Escherichia coli*[1,2,3] and *Pseudomonas putida*[4] has recently been shown. In this presentation, we report on the metabolic engineering of *Pseudomonas taiwanensis* VLB120[5] for the production of saturated and monounsaturated medium chain methyl ketones (C11, C13, C15, C17). Major arguments for the use of this microbe are its metabolic versatility, high tolerance of organic solvents[5] and ease of cultivation. *P. taiwanensis* VLB120 can grow on various carbon sources besides glucose such as glycerol, an important by-product of biodiesel production, as well as on major components of biomass hydrolysate such as xylose, organic acids and aromatic compounds, e.g., 4-hydroxybenzoate[4]. Further, its superior redox cofactor regeneration capability[6] might benefit the synthesis of the reduced, aliphatic target compounds. The transformation of *P. taiwanensis* VLB120 into a microbial cell factory for methyl ketone production was achieved by: (i) overproduction of the fatty acyl-CoA synthetase FadB to increase acyl-CoA availability, (ii) oxidation of acyl-CoA to a trans-2-enoyl-CoA by a heterologously expressed acyl-CoA oxidase from *Micrococcus luteus*, (iii) conversion of this intermediate to β-hydroxyacyl-CoA and further oxidation to a β -ketoacyl-CoA by overexpression of the native *fadB* gene, (iv) increased thioesterase activity by overexpression of *fadM* to form free β -keto acids, which spontaneously decarboxylate to methyl ketones. The 1st generation production strain yielded 550 mg L^{-1}_{aq} methyl ketones in a batch fermentation with *in situ* product extraction into a second organic layer of *n*-decane. Further strain optimization was guided by metabolic modeling, which suggested an additional deletion of the acyl-CoA thioesterase II (*tesB*). TesB hydrolyzes acyl-CoA to free fatty acids, hence, reverses the desired FadD reaction. In a simple batch fermentation, the proposed gene deletion resulted in a 2.5-fold increased product titer of 1.4 g L^{-1}_{aq} while 9.4 g L^{-1}_{aq} were reached in fed-batch cultivations. Additional, successful strategies tested in parallel were the deletion of the *pha* operon, responsible for polyhydroxyalkanoate synthesis and deletion of a *fadA* homologue in the 1st generation production strain, with the later resulting in an even 4-fold improvement of the product titer. While the production of 9.4 g L^{-1}_{aq} is already the highest reported titer of recombinantly produced methyl ketones so far, consolidation of all successfully tested engineering strategies holds great promise to significantly boost methyl ketone production in *P. taiwanensis* VLB120 to even higher titers. Overall, the results of this study underline the high potential of *P. taiwanensis* VLB120 for the production of methyl ketones and highlight model-guided metabolic engineering as a means to rationalize and accelerate strain optimization efforts.

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[2]Goh et al. 2012: doi: 10.1128/AEM.06785-11
[3]Goh et al. 2014: doi: 10.1016/j.ymben.2014.09.003.
[4]Goh et al. 2018: doi: 10.1002/bit.26558.
[5]Rühl et al. 2009: doi: 10.1128/AEM.00225-09
[6]Blank et al. 2008: doi: 10.1111/j.1742-4658.2008.06648.x.