METABOLIC ENGINEERING OF *PSEUDOMONAS PUTIDA* KT2440 FOR ENHANCED RHAMNOLIPID PRODUCTION

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The production of chemicals and fuels is mainly based on fossil resources. The reduced availability of these resources and thus the increasing prices for crude oil as well as the resulting pollution of the environment require alternative strategies to be developed. One approach is the employment of microorganisms for the production of platform molecules using renewable resources as substrate. Biosurfactants, such as rhamnolipids, are an example for such products as they can be naturally produced by microorganisms and are biodegradable in contrast to chemical surfactants. The bio-based production of chemicals has to be efficient and sustainable to become competitive on the market. Several strategies can be applied to increase the efficiency of a microbial cell factory, e.g., streamlining the chassis. Here, we show the heterologous production of rhamnolipids with the non-pathogenic *Pseudomonas putida* KT2440 with the aim of increasing the yield. *P. putida* KT2440 is a well-characterized microorganism and its genome is sequenced and well annotated. Thus, the targeted removal of genes is possible and can lead to a reduction of the metabolic burden and by-product formation, which can result in a higher yield. Furthermore, the efficient supply of precursors is an important factor for optimized production processes. Rhamnolipids are amphiphilic molecules containing rhamnose and ß-hydroxy fatty acids. These precursors are synthesized by two pathways, the fatty acid *de novo* synthesis and the rhamnose pathway. We performed gene deletions to avoid the synthesis of by-products, like pyoverdine, exopolysaccharides, and large surface proteins and energy consuming devices as the flagellum. Most of the genome-reduced mutants reached a higher yield compared to the strain with wildtype background. With the best chassis, the yield could be increased by 35%. Furthermore, we conducted the overexpression of genes for precursor supply, either plasmid-based or genomically integrated. In this regard, the genes for the phosphoglucomutase, the complete rhamnose-synthesis pathway operon, and different enzymes in the pathway for acetyl-CoA synthesis were targeted. Various combinations were tested, and the highest yield reached was 51% higher compared to the initial rhamnolipid producer. Finally, a genome-reduced mutant was equipped with the overexpression modules and the rhamnolipid titer was increased from approximately 590 mg/L for the wildtype background to 960 mg/L, which represents a 63% increase. In conclusion, we were able to enhance the yield of rhamnolipids per glucose using metabolic engineering.