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COMMENTARY

Fully glycerol-independent microbial production of 1,3-propanediol via non-natural pathway: Paving the way to success with synthetic tiles

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See accompanying article by Zhen Chen et al. DOI: 10.1002/biot.201400235

A number of inventive genetic and metabolic engineering strategies towards improved 1,3-propanediol (1,3-PDO) production have been reported to date. So far, all of the strategies relied on bioconversion of glycerol. Either glycerol has been used as a primary substrate in the culture medium, or glycerol was an intermediate in a synthetic pathway leading from glucose to 1,3-PDO. The article by Chen et al. [1] in this issue of *Biotechnology Journal* constitutes a paradigm shift in our understanding of microbial 1,3-PDO production. The authors constructed a fully glycerol-independent pathway of 1,3-PDO synthesis from glucose by recruiting native metabolites of central carbon and nitrogen metabolism. The connection between the native and heterologous parts was secured by an engineered protein with modified characteristics, which constitutes a substantial added value of this contribution. The paper illustrates an excellent example of a successful synthetic biology approach with application in industrial biotechnology.

Glycerol is the conventional substrate for 1,3-PDO production, nevertheless, efforts towards development of alternative, non-natural routes of 1,3-PDO synthesis have been pursued. In general, three different strategies can be adopted: i) engineering natural 1,3-PDO producers, to endow them with the ability to produce glycerol from glucose; ii) extending the glycerol synthesis pathway towards 1,3-PDO production in good glycerol-producers; iii) establishment of the whole pathway from glucose to 1,3-PDO in microbes that possess neither of these traits. Until now, 1,3-PDO synthesis was accomplished through heterologous expression of two panels of genes involved in glucose-to-glycerol and glycerol-to-1,3-PDO bioconversion (Figure 1).

The first strategy, engineering natural 1,3-PDO producers, to endow them with the ability to produce glycerol from glucose, is the least explored one. Production of 1,3-PDO from sugar could be accomplished in native 1,3-PDO producers by expression of glycerol-3-phosphate phosphatase (G3PdeP), which diverts intermediates of central carbon metabolism towards glycerol production. To date, however, no significant progress involving the strategy to express G3PdeP in native 1,3-PDO producers has been reported. On the other hand, several articles on extending the glycerol synthesis pathway towards 1,3-PDO production in good glycerol-producers have been published [2–7]. *Saccharomyces cerevisiae* strains harboring enterobacterial genes involved in 1,3-PDO formation could produce 1,3-PDO at relatively low titers (0.4 g/L [2], 1.2 g/L [3]). Non-conventional yeasts, *Hansenula polymorpha* and *Zygosaccharomyces rouxii*, also served as hosts for the 1,3-PDO-synthesis genes from *Klebsiella pneumoniae*, resulting in recombinant strains capable of 1,3-PDO formation through the native glycerol intermediate, with the final titer of 2.4 g/L [4] and 17.1 g/L [5], respectively. The most notable achievement in biotechnological 1,3-PDO production from glucose was reported by DuPont and Genencor [6] and falls into the third category, i.e. establishment of the whole pathway from glucose to 1,3-PDO in microbes that possess neither of these traits. The successful metabolic engineering strategy, resulting in 135 g/L of 1,3-PDO, included i.a. overexpression of heterologous genes (glucose–glycerol–1,3-PDO), re-direction of carbon fluxes, and modification of the glucose transportation system. The final engineered *Escherichia coli* strain now operates in a commercial process [6]. The strategy by DuPont and Genencor was later partially followed by Liang et al. [7], resulting in a recombinant *E. coli* strain, expressing the heterologous genes, able to synthesize 1,3-PDO from glucose at a titer of 12.1 g/L.

The current report by An-Ping Zeng and co-workers [1] presents a completely new strategy of genetic engineering for microbial 1,3-PDO production. The concept relies on channeling the carbon flux from glucose through the homoserine synthesis pathway towards 1,3-PDO formation via three heterologous enzymatic activities. Importantly, as no natural enzyme is able to catalyze the first reaction of homoserine deamination

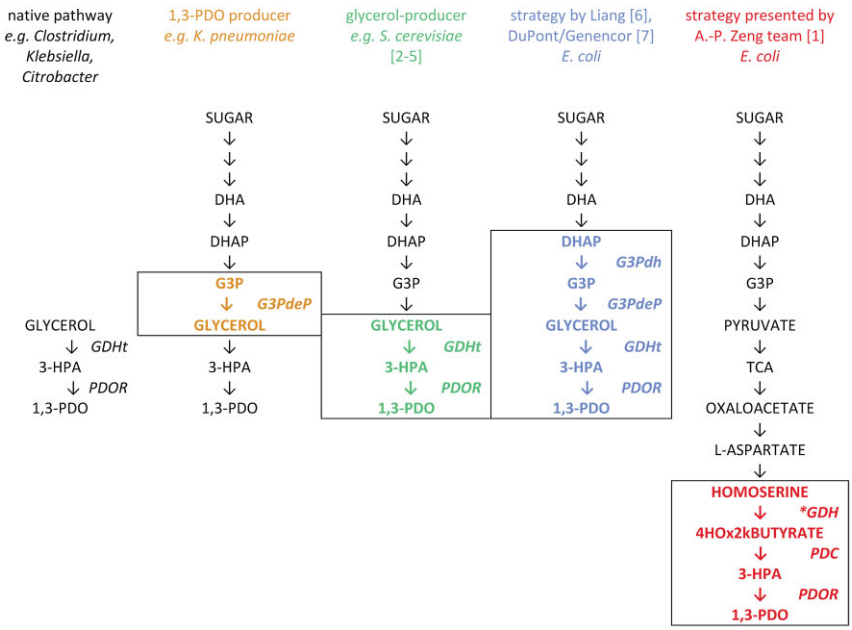

Figure 1. General strategies followed for establishment of glucose-to-1,3-PDO pathway. The framed and colored part of the pathway is the one modified in the respective strategy. Abbreviations: metabolites and pathways: 3-HPA – 3-hydroxypropionaldehyde, 1,3-PDO – 1,3-propanediol, DHA – dihydroxyacetone, DHAP – dihydroxyacetone phosphate, G3P – glycerol-3-phosphate, TCA – tricarboxylic acid cycle, 4-HOx-2-kBUTYRATE – 4-hydroxy-2-ketobutyrate; enzymes (provided in italics): *GDHt* – glycerol dehydratase (most frequently used *dhaB1-4* genes from *K pneumoniae*), *PDOR* – 1,3-propanediol oxidoreductase (most frequently used *dhaT* gene from *K. pneumoniae* or *yqhD* from *E. coli*), *G3PdeP* – glycerol-3-phosphate phosphatase from *S. cerevisiae*, *G3Pdh* – glycerol-3-phosphate dehydrogenase from *S. cerevisiae*, **GDH* – engineered glutamate dehydrogenase, *PDC* – pyruvate decarboxylase from *Zymomonas mobilis*.

to generate 4-hydroxy-2-ketobutyrate, the authors engineered glutamate dehydrogenase and changed its substrate specificity. The novelty of this report [1] lies in the establishment of a new operable pathway for 1,3-PDO synthesis with full independence from glycerol, but also without requirement for Co-B12 or SAM cofactors (essential for glycerol dehydratase activity, GDHt, involved the native pathway). As the authors mentioned, at this moment the manuscript constitutes a "proof-of-concept", due to a relatively low 1,3-PDO titer (51.5 ± 4.9mg/L) and requirement for external homoserine addition, but definitely it is an original and valuable contribution with great potential for further development.

The author declares no financial or commercial conflict of interest.

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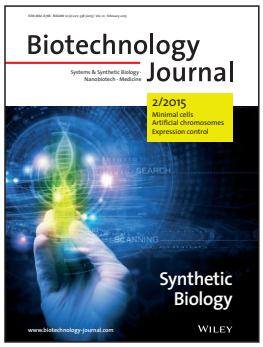

Special issue: Synthetic Biology.
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