

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

Ewelina Celińska

Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, Poznan, Poland



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

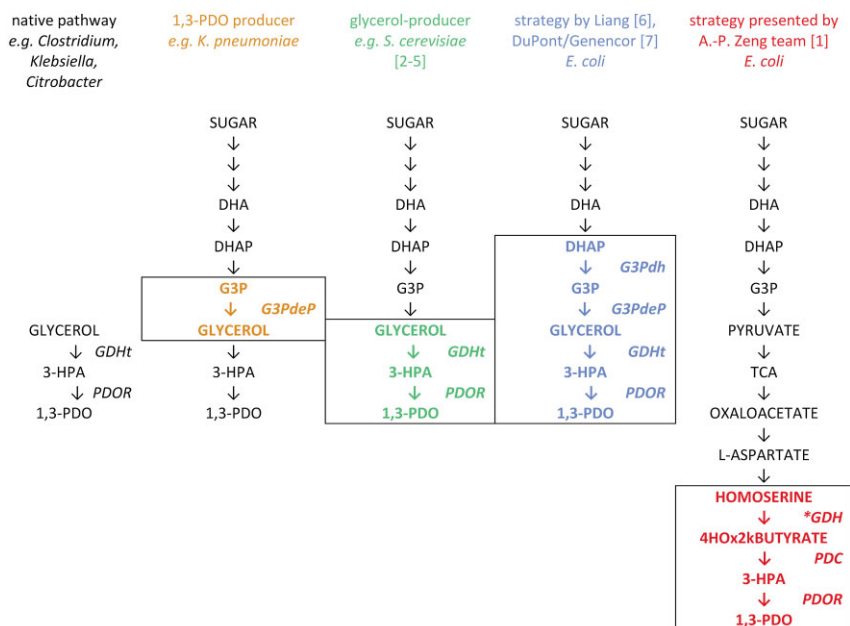


Figure 1. General strategies followed for establishment of glucose-to-1,3-PDO pathway. The framed and colored part of the pathway is the one modified in the respective strategy. Abbreviations: metabolites and pathways: 3-HPA – 3-hydroxypropionaldehyde, 1,3-PDO – 1,3-propanediol, DHA – dihydroxyacetone, 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 ± 4.9 mg/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.

References

- [1] Chen, Z., Geng, F., Zeng, A.-P., Protein design and engineering of a de novo pathway for microbial production of 1,3-propanediol from glucose. *Biotechnol. J.* 2014, DOI: 10.1002/biot.201400235
- [2] Rao, Z., Ma, Z., Shen, W., Fang, H. et al., Engineered *Saccharomyces cerevisiae* that produces 1,3-propanediol from D-glucose. *J. Appl. Microbiol.* 2008, 105, 1768–1776.
- [3] Ma, Z., Rao, Z., Xu, L., Liao, X. et al., Expression of *dha* operon required for 1,3-PD formation in *Escherichia coli* and *Saccharomyces cerevisiae*. *Curr. Microbiol.* 2010, 60, 191–198.
- [4] Hong, W. K., Kim, C. H., Heo, S. Y., Luo, L. H. et al., 1,3-Propanediol production by engineered *Hansenula polymorpha* expressing *dha* genes from *Klebsiella pneumoniae*. *Bioprocess. Biosyst. Eng.* 2011, 34, 231–236.
- [5] Ma, Z., Bian, Y., Shentu, X., Yu, X., Development of a novel recombinant strain *Zygosaccharomyces rouxii* JL2011 for 1,3-propanediol production from glucose. *Appl. Microbiol. Biotechnol.* 2013, 97, 4055–4064.
- [6] Nakamura, C.E., Whited, G.M., Metabolic engineering for the microbial production of 1,3-propanediol. *Curr. Opin. Biotechnol.* 2003, 14, 454–459.
- [7] Liang, Q., Zhang, H., Li, S., Qi, Q., Construction of stress-induced metabolic pathway from glucose to 1,3-propanediol in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 2011, 89, 57–62.

Correspondence: Dr. Ewelina Celińska
E-mail: celinska@up.poznan.pl



Special issue: Synthetic Biology.

Synthetic biology has become more application oriented, by designing and implementing synthetic pathways in industrial biotechnology. This Special issue, edited by Roland Eils (German Cancer Research Center, DKFZ, and University of Heidelberg), Julia Ritzerfeld (German Cancer Research Center, Heidelberg) and Wolfgang Wiechert (IBG-1: Biotechnology, Forschungszentrum Jülich), includes contributions from the Helmholtz Initiative on Synthetic Biology and focusses on applications of synthetic biology in biotechnology.

Image: © Sergey Nivens Fotolia.com

Biotechnology Journal – list of articles published in the February 2015 issue.

Editorial: Synthetic biology – ready for application

Roland Eils, Julia Ritzerfeld, Wolfgang Wiechert

<http://dx.doi.org/10.1002/biot.201400842>

Forum

Synthetic biology's self-fulfilling prophecy – dangers of confinement from within and outside

Daniel Frank, Reinhard Heil, Christopher Coenen, Harald König

<http://dx.doi.org/10.1002/biot.201400477>

Forum

Synthetic biology and intellectual property rights: Six recommendations

Timo Minssen, Berthold Rutz, Esther van Zimmeren

<http://dx.doi.org/10.1002/biot.201400604>

Commentary

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

Ewelina Celinska

<http://dx.doi.org/10.1002/biot.201400360>

Commentary

Chassis organism from *Corynebacterium glutamicum*: The way towards biotechnological domestication of *Corynebacteria*

Victor de Lorenzo

<http://dx.doi.org/10.1002/biot.201400493>

Review

RNA aptamers as genetic control devices: The potential of riboswitches as synthetic elements for regulating gene expression

Christian Berens, Florian Groher and Beatrix Suess

<http://dx.doi.org/10.1002/biot.201300498>

Review

CRISPR genome engineering and viral gene delivery: A case of mutual attraction

Florian Schmidt and Dirk Grimm

<http://dx.doi.org/10.1002/biot.201400529>

Review

Optogenetic control of signaling in mammalian cells

Hannes M. Beyer, Sebastian Naumann, Wilfried Weber and Gerald Radziwill

<http://dx.doi.org/10.1002/biot.201400077>

Rapid Communication

Protein design and engineering of a de novo pathway for microbial production of 1,3-propanediol from glucose

Zhen Chen, Feng Geng and An-Ping Zeng

<http://dx.doi.org/10.1002/biot.201400235>

Research Article

Chassis organism from *Corynebacterium glutamicum* – a top-down approach to identify and delete irrelevant gene clusters

Simon Unthan, Meike Baumgart, Andreas Radek, Marius Herbst, Daniel Siebert, Natalie Brühl, Anna Bartsch, Michael Bott, Wolfgang Wiechert, Kay Marin, Stephan Hans, Reinhard Krämer, Gerd Seibold, Julia Frunzke, Jörn Kalinowski, Christian Rückert, Volker F. Wendisch and Stephan Noack

<http://dx.doi.org/10.1002/biot.201400041>

Research Article

Synthetic secondary chromosomes in *Escherichia coli* based on the replication origin of chromosome II in *Vibrio cholerae*

Sonja J. Messerschmidt, Franziska S. Kemter, Daniel Schindler and Torsten Waldminghaus

<http://dx.doi.org/10.1002/biot.201400031>

Research Article

Bacterial XylRs and synthetic promoters function as genetically encoded xylose biosensors in *Saccharomyces cerevisiae*

Wei Suong Teo and Matthew Wook Chang

<http://dx.doi.org/10.1002/biot.201400159>

Biotech Method

Single-cell analysis reveals heterogeneity in onset of transgene expression from synthetic tetracycline-dependent promoters

Ulfert Rand, Jan Riedel, Upneet Hillebrand, Danim Shin, Steffi Willenberg, Sara Behme, Frank Klawonn, Mario Köster, Hansjörg Hauser and Dagmar Wirth

<http://dx.doi.org/10.1002/biot.201400076>

Biotech Method

Engineering connectivity by multiscale micropatterning of individual populations of neurons

Jonas Albers, Koji Toma and Andreas Offenhäusser

<http://dx.doi.org/10.1002/biot.201400609>