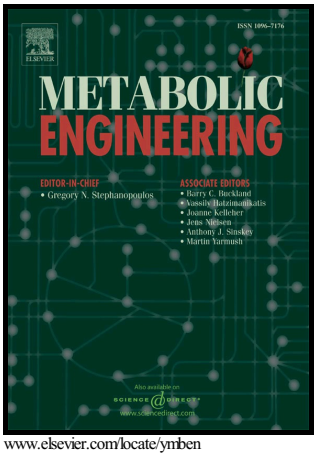

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Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization

Mary Ann Franden[a 1], Lahiru Jayakody[a1], Wing-Jin Li[b], Neil J. Wagner [a], Nicholas S. Cleveland[a], William E. Michener[a], Bernhard Hauer[c], Lars M. Blank[b], Nick Wierckx[b*], Janosch Klebensberger[c*], and Gregg T. Beckham[a*]

[a] National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA
[b] Institute of Applied Microbiology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany
[c] University of Stuttgart, Institute of Biochemistry and Technical Biochemistry, Allmandring 31, 70569 Stuttgart, Germany
nick.wierckx@rwth-aachen.de

janosch.klebensberger@itb.uni-stuttgart.de

gregg.beckham@nrel.gov

[*]Corresponding Author Information:

ABSTRACT
Ethylene glycol is used as a raw material in the production of polyethylene terephthalate, in antifreeze, as a gas hydrate inhibitor in pipelines, and for many other industrial applications. It is metabolized by aerobic microbial processes via the highly toxic intermediates glycolaldehyde and glycolate through C2 metabolic pathways. *Pseudomonas putida* KT2440, which has been engineered for environmental remediation applications given its high toxicity tolerance and broad substrate specificity, is not able to efficiently metabolize ethylene glycol, despite harboring putative genes for this purpose. To further expand the metabolic portfolio of *P. putida*, we elucidated the metabolic pathway to enable ethylene glycol via systematic overexpression of glyoxylate carboligase (*gcl)* in combination with other genes. Quantitative reverse transcription polymerase chain reaction demonstrated that all of the four genes in genomic proximity to *gcl* (*hyi*, *glxR*, *ttuD*, and *pykF*) are transcribed as an operon. Where the expression of only two genes (*gcl* and *glxR*) resulted in growth in ethylene glycol, improved growth and ethylene glycol utilization were observed when the entire *gcl* operon was expressed. Both glycolaldehyde and glyoxal inhibit growth in concentrations of ethylene glycol above 50 mM. To overcome this bottleneck, the additional overexpression of the glycolate oxidase (*glcDEF*) operon removes the glycolate bottleneck and minimizes the production of these toxic intermediates, permitting growth in up to 2 M (~124 g/L) and complete consumption of 0.5 M (31 g/L) ethylene glycol in shake flask experiments. In addition, the engineered strain enables conversion of ethylene glycol to medium-chain-length polyhydroxyalkanoates (mcl-PHAs). Overall, this study provides a robust *P. putida* KT2440 strain for ethylene glycol consumption, which will serve as a foundational strain for further biocatalyst development for applications in the remediation of waste polyester plastics and biomass-derived wastewater streams.

[1] Denotes equal contribution