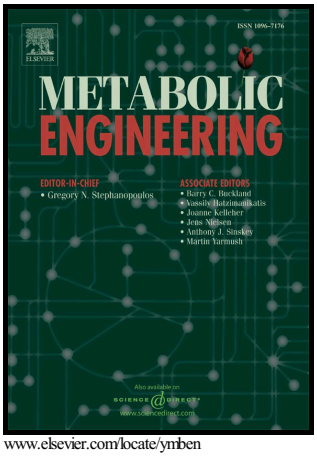

www.elsevier.com/locate/ymben

Metabolic Engineering of *Pseudomonas taiwanensis* VLB120 with Minimal Genomic Modifications for High-Yield Phenol Production

Benedikt Wynands, Christoph Lenzen, Maike Otto, Falk Koch, Lars M. Blank, Nick Wierckx

PII: S1096-7176(17)30448-2
DOI: https://doi.org/10.1016/j.ymben.2018.03.011
Reference: YMBEN1365

To appear in: *Metabolic Engineering*

Received date: 1 December 2017
Revised date: 7 March 2018
Accepted date: 11 March 2018

Cite this article as: Benedikt Wynands, Christoph Lenzen, Maike Otto, Falk Koch, Lars M. Blank and Nick Wierckx, Metabolic Engineering of *Pseudomonas taiwanensis* VLB120 with Minimal Genomic Modifications for High-Yield Phenol Production, *Metabolic Engineering,* https://doi.org/10.1016/j.ymben.2018.03.011

Metabolic Engineering of *Pseudomonas taiwanensis* VLB120 with Minimal Genomic Modifications for High-Yield Phenol Production

Benedikt Wynands, Christoph Lenzen, Maike Otto, Falk Koch, Lars M. Blank, and Nick Wierckx*

Institute of Applied Microbiology, RWTH Aachen University, Worringerweg 1, 52074 Aachen

*: corresponding author,

Nick Wierckx, Institute of Applied Microbiology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany, e-mail: nick.wierckx@rwth-aachen.de, phone: +49 241 80 266 49

Abstract

Aromatic chemicals are important building blocks for the production of a multitude of everyday commodities. Currently, aromatics production relies almost exclusively on petrochemical processes. To achieve sustainability, alternative synthesis methods need to be developed. Here, we strived for an efficient production of phenol, a model aromatic compound of industrial relevance, from renewable carbon sources using the solvent-tolerant biocatalyst *Pseudomonas taiwanensis* VLB120. First, multiple catabolic routes for the degradation of aromatics and related compounds were inactivated, thereby obtaining the chassis strain *P. taiwanensis* VLB120Δ5 incapable of growing on 4-hydroxybenzoate (Δ*pobA*), tyrosine (Δ*hpd*), and quinate (Δ*quiC*, Δ*quiC1*, Δ*quiC2*). In this context, a novel gene contributing to the quinate catabolism was identified (*quiC2*). Second, we employed a combination of reverse- and forward engineering to increase metabolic flux towards the product, using leads obtained from the analysis of aromatics producing *Pseudomonas putida* strains previously generated by mutagenesis. Phenol production was enabled by the heterologous expression of a codon-optimized and chromosomally integrated tyrosine phenol-lyase encoding gene from *Pantoea agglomerans* AJ2985 (*PaTPL2*). The genomic modification of endogenous genes encoding $TrpE^{P290S}$,