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Rational and Evolutionary Engineering of Industrial *Saccharomyces Cerevisiae* Strains for Production of Chemicals from Xylose-Rich Feedstocks

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Biorefineries have been developed for industrial scale conversion of renewable biomass to gas, energy and various chemicals as an alternative to oil-based petrochemical industry. The biorefinery concept relies on robust and efficient microbial cell factories. Yeast *Saccharomyces cerevisiae* is one of the most used platform cell factories in the existing biorefineries. Second generation biorefineries process non-food agricultural or forest biomass and here efficient utilization of both C6 and C5 sugars is crucial. In this work we aim at developing of novel robust yeast cell factories for production of selected dicarboxylic acids that can be polymerised into bio-based polymers, from pentose-rich lignocellulose hydrolysates. Industrial yeast strains that are able to cope with the harsh environment in industrial settings have been selected as the production organism. We developed a molecular approach based on CRISPR-Cas9 (Stovicek et al., *Metab Eng Commun* 2015) for fast construction of gene disruptions and second generation of EasyClone vectors suitable for delivery and stable integration of genes into the genome of industrial yeast strains (Stovicek et al., *J Ind Microbiol* 2015). Such tools allow us to overcome genetic complexity of industrial strains and simplify the strain construction. Using the developed tools and adaptive evolution we constructed efficient xylose-fermenting industrial yeast strain performing in various media. Relevant metabolic and evolutionary engineering strategies will be discussed. This project is part of BioREFINE-2G (www.biorefine2g.eu), which is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-613771).