EVALUATION OF EFFICIENT METABOLIC ENGINEERING STRATEGIES FOR ACCELERATED CO-UTILISATION OF LACTOSE BY SACCHAROMYCES CEREVISIAE

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Body

Cheese whey is the by-product of cheese production in the dairy industry and its disposal represents an environmental concern. Considering the need to implement circular bioeconomy systems, cheese whey surplus can be reused as a substrate to generate value-added compounds. Due to its high lactose content, whey is successfully integrated in multi-waste biovalorisation approaches, increasing sugar content of lignocellulose substrates[1]. In this context, the ability of co-metabolization of glucose and lactose, together with yeast robustness and inhibitor tolerance, will be pivotal to develop and implement such sustainable bioprocesses. The yeast *Saccharomyces cerevisiae* is an attractive industrial cell factory reported to produce several chemicals of interest, from biofuels to high-value bioproducts, but it is not able to utilize lactose as carbon source. To surpass this hindrance, strategies like cloning *LAC4* (β-galactosidase) and *LAC12* genes (lactose permease) from *Kluyveromyces* sp. have been applied, but complete lactose metabolization by recombinant strains is known to be an issue[2]. Since then, several different approaches have been studied for an efficient lactose utilisation, as increasing the copy number of lactose-metabolization genes. However, to ensure genomic stability, it would be preferable to limit the copy number and regulate differentially the expression of both genes. This work spans the valorisation of cheese whey effluent using yeasts as metabolic factories. A host able to co-metabolise glucose and galactose was used, as the choice of the yeast chassis is determinant in metabolic engineering outcomes[3]. Through the CRISPR/Cas9 system, an industrial *S. cerevisiae* strain was successfully engineered with the *LAC4* and *LAC12* genes under the control of different promoters. The effects of its combination were considered and the better ratio between β-galactosidase and lactose permease was evaluated. Physiological characterization of the strains constructed was performed and optimal conditions for lactose metabolization were determined. All strains studied were capable of consuming all lactose in the media, with the top-producing strains achieving yields near the theoretical maximum in both synthetic media and cheese whey fermentations. This study constitutes an important basis for the insertion of other metabolic pathways allowing the production of value-added compounds using cheese whey by-product as substrate and/or co-substrate.**Acknowledgements**

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