

## Engineering of robust yeast for valorisation of coffee industry wastes

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*Saccharomyces cerevisiae* PE-2, one of the most robust yeast chassis for use in second-generation bioprocesses [1-2], is incapable of utilizing galactose, which is abundant in spent coffee grounds (SCG), the solid fraction wastes deriving from coffee industry. In this study, homology-based analysis of the Leloir pathway of the *S. cerevisiae* JAY291 (haploid derivative of the strain PE-2) allowed the identification of 11 amino acid (aa) substitutions in the Gal2 sequence that are not conserved across other industrial and laboratorial strains. Four of these point mutations were found within or in the vicinities of the transmembrane domain 7 (TM7), a region important for substrate recognition [3]. Among these, a significant substitution includes the F336L, as the loss of certain aromatic aa in TM7 was reported to be critical for galactose transport activity [3]. Since these results suggested that the galactose permease of *S. cerevisiae* PE-2 might lack galactose transport activity, this strain was transformed with a plasmid containing the CEN.PK113-5D GAL2 under the regulation of the TDH3 promoter and PGI1 terminator. The resulting transformants were physiologically characterized in liquid YP containing 2% galactose. Expression of the CEN.PK113-5D GAL2 in PE-2 established its galactose utilization capacity. The new strain was then evaluated in hydrolysate obtained by acid hydrolysis of SCG, which is predominantly composed by mannose and galactose. The engineered PE-2 efficiently utilized the galactose from SCG hydrolysate, being faster than CEN.PK113-5D in consuming all the available sugars in this hydrolysate. These results open new perspectives and opportunities for the valorisation of galactose-containing second-generation substrates by this newly constructed robust strain.

1. Bioresour Technol (2015) 191:7-16
2. Bioresour Technol (2017) 227:24-34
3. J Biol Chem (2000) 11:4422-28