Reference

219

Production of bioethanol from concentrated cheese whey lactose using flocculent *Saccharomyces cerevisiae*

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Abstract

The lactose in cheese whey (the main by-product of dairy industries) is an interesting substrate for fermentation processes, particularly for the production of bulk commodities such as bioethanol, due to the large amounts of whey surplus produced globally. Whey fermentation yields potable ethanol that can be used not only for fuels but also in food and beverage industries. Since most lactose-consuming microorganisms do not present physiological characteristics suitable for ethanol production bioprocesses, the construction of genetically engineered *Saccharomyces cerevisiae* (wild strains are lactose-negative) strains able to ferment lactose has been envisaged.

Our group has constructed a lactose-fermenting *S. cerevisiae* strain expressing the *LAC12* (permease) and *LAC4* (beta-galactosidase) genes of *Kluyveromyces lactis*, using a highly flocculent strain (NCYC869) as host for transformation. The properties of the original recombinant (T1) were further improved using evolutionary engineering approaches, which yielded an evolved strain (T1-E) that fermented lactose faster with higher ethanol yield and with improved flocculation (Guimarães et al., 2008, Appl Environ Microbiol 74: 1748-56).

In shake-flask fermentations with concentrated whey containing 150 g/L initial lactose, the evolved strain was unable to completely consume the lactose (lactose residual > 35 g/L) producing 6% (v/v) ethanol. Supplementation of the whey with 10 g/L of corn steep liquor (CSL) enhanced lactose consumption (residual < 3 g/L) and increased the ethanol titre to 8% obtained after 42 h of fermentation, which corresponds to an ethanol productivity of > 1.5 g/L/h. The evolved strain has further been tested in a 6 L air-lift bioreactor, producing over 6% ethanol from concentrated whey (110-120 g/L lactose) supplemented with 10 g/L CSL. Taking advantage of flocculation, yeast biomass was easily recycled by sedimentation for repeated-batch operation, allowing accumulation of high cell densities in the bioreactor. The yeast biomass was active for over 5 consecutive fermentations during which viability (methylene blue staining) was > 95%. The ability of the evolved recombinant to ferment concentrated cheese whey lactose together with its flocculation characteristics have the potential to greatly enhance the economical viability of whey-to-ethanol conversion processes.