

Engineering yeasts and fermentation processes for bioethanol production

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Energy crisis and environmental concerns are increasingly making bioethanol an attractive renewable fuel source. Our aim is to develop yeast (*Saccharomyces cerevisiae*) strains and fermentation media (based on industrial substrates/by-products) that enable to attain high fermentation rates and high ethanol titres at the end of the fermentation process, therefore minimizing distillation costs (which are considered a major constraint in industrial bioethanol production). We have studied fermentation kinetics of four *S. cerevisiae* strains in a medium containing 300 – 350 g/L glucose with 100 g/L of corn steep liquor (CSL) as the sole nutrient source, using Erlenmeyer flasks fitted with glycerol-locks. The two laboratory strains tested, CEN.PK 113-7D and NCYC 869 (highly flocculent), yielded final ethanol titres of 16.8 ± 0.1 % (v/v) and 12.8 ± 0.1 % (v/v), respectively. Under the same fermentation conditions, strains CA-116 and RL-11, which were isolated from "cachaça" production in Brazil, were able to produce 16.4 ± 0.1 % (v/v) and 17.1 ± 0.1 % (v/v), respectively. However, using a medium with such high CSL concentration (100 g/L) could compromise the economical viability of industrial fermentation process. Thus, using factorial design approaches we intend to partially replace CSL with other cheap nutrient sources in order to optimize the ethanol productivity and reduce the medium costs. For this optimization process we are using a basic medium consisting of 300 g/L glucose syrup and 15 g/L CSL. So far, our results with strain CEN.PK 113-7D showed that supplementation of this medium with 1.5 g/L urea strongly increased the fermentation rate as well as the final ethanol concentration (which was 12.8 % v/v using the basic medium without supplementation and 17.1 % v/v with urea supplementation). Moreover, supplementation of the basic medium with mineral salts, particularly magnesium and calcium salts, also induced significant improvements in the fermentation rate and final ethanol titres. Additionally, we are currently devising evolutionary engineering strategies in order to select mutants of the aforementioned strains with higher tolerance to high sugar (glucose) and ethanol stresses, which may prove useful for industrial bioethanol processes.