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"Gheorghe Asachi" Technical University of Iasi, Romania

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METABOLIC ENGINEERING IN *Enterococcus faecalis* FOR ITS USE IN BIO-ETHANOL PRODUCTION FROM WHEY

N.F. Rana, S. Gente, J.M. Laplace, Y. Auffray

USC INRA 2017 Laboratoire de Microbiologie de l'Environnement, Université de Caen Basse-Normandie Espalande de la Paix 14032 Caen cedex, France

Abstract

Lactose is an interesting carbon source for the production of several bio-products by fermentation, primarily because it is the major component of cheese whey, the main by-product of dairy activities. However, the yeast *Saccharomyces cerevisiae*, the microorganism more widely used in industrial fermentation processes, does not have a lactose metabolization system. Thus, strain development programs through metabolic engineering are required for the implementation of lactose-to-ethanol processes with increased productivity. In this study the possibility of using whey permeate as a raw material for the production of ethanol using *Enterococcus faecalis* was examined. *Enterococcus faecalis* is a lactic acid bacterium that metabolizes many carbon sources and tolerates harsh environmental conditions. This study presents the potential of genetically modified *E. faecalis* JH2-2 strain for the production of bioethanol from glucose and lactose. *E. faecalis* has two lactate dehydrogenase genes (*ldh*) involved in lactate production. The deletion of these *ldh* genes led to increased production of formate, acetate and ethanol. The wild type JH2-2 converts only 4,8 % of the sugar to ethanol, but after the deletion of *ldh* genes this level is increased up to 15,2 %. To enhance ethanol production ability of *E. faecalis* mutant Δ*ldh1*/Δ*ldh2*, a pyruvate decarboxylase gene from *Clostridium acetobutylicum* was inserted in its genome under the control of the strong *ldh1* promoter. The recombinant PDC was found to have specific activity of 17 U/mg and ethanol yield was increased to 19 %. Moreover the induced PDC expression was also observed in the *ldh* deficient mutant Δ*ldh1*/Δ*ldh2* with an expression vector pMSP3535 inducible with nisin. Using this strategy, the specific activity of the PDC was found to be 47 U/mg with an ethanol yield of 40 %. Transcriptional analysis of *E. faecalis* JH2-2 and its mutants by a qRT-PCR approach reveals that the deficiency of the lactate dehydrogenase resulted in the up-regulation of the genes involved in the production of formate and acetate. Whereas the genes encoding enzymes implicated in acetoin production are down-regulated. Out of four *adh* genes in *E. feacalis* only *adhE* and *adhZn* are expressed. This study presents a successful cloning of the *pdc* gene of C. *acetobutylicum* in *E. faecalis* and it also reveals the efficiency of molecular modifications for the use of *E. faecalis* for bio-ethanol production. Further optimization and modifications can make a promising combination of genetically modified *E. faecalis* for the production of ethanol from waste materials such as whey.