Biocatalysis & Biotransformation

P002

ENHANCEMENT OF CASTOR OIL BIOTRANSFORMATION INTO AROMA BY *YARROWIA LIPOLYTICA* MUTANTS

Adelaide Braga[1]; Isabel Belo[1]

[1] *IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho*

The food industry has a great interest in biotechnological production of ɣ-decalactone by *Yarrowia lipolytica*, due to its increasing consumers acceptability in comparison with similar products obtained by chemical synthesis. This yeast is able to produce ɣ-decalactone by transformation of a hydroxylated C_{18} fatty acid. However, lower yields of ɣ-decalactone were obtained (up to 4-5 gL^{-1}), mainly due the degradation of newly synthesized lactone and the partial use of ricinoleic acid or intermediate at the C_{10} level, which is simultaneously the precursor for other ɣ-lactones.

Thus, the purpose of this work is to enhance the biotransformation of castor oil, source of ricinoleic acid, into ɣ-decalactone exploring different operation mode strategies in bioreactor (batch and fed-batch) and compare the yields obtained with wild type strain with those achieved by mutant strains.

Different experiments were conducted in a 3.7-L bioreactor using an aeration rate of 5.1 L min^{-1}, agitation 650 rpm and pH 6.0 (previously optimized conditions [1]). The influence of castor oil concentration and cell density on ɣ-decalactone production was investigated. Two different cell and castor oil concentrations (30 g L^{-1} and 60 gL^{-1}) were used for the biotransformation. In the expectation of achieving higher ɣ-decalactone concentrations, a step-wise fed-batch strategy was also attempted.

In a first approach, this study was conducted with *Yarrowia lipolytica* W29 (ATCC20460) and the highest ɣ-decalactone productivity of 215.4 mg L^{-1} h^{-1} was obtained in a batch mode of operation with 60 g L^{-1} of cells and 60 g L^{-1} of castor oil. After that, ɣ-decalactone production with two *Yarrowia lipolytica* mutants was studied. Experiments performed with *Y. Lipolytica* MTLY40-2P, with a deletion of all the POX 3-5 genes and a multicopy insertion of *POX2* [2], resulted in an increased accumulation and an inhibition of ɣ-decalactone degradation. Since this yeast is also known to be a lipase producer and these enzymes catalyze the hydrolysis of triacylglycerides into glycerol and free fatty acids, a *Y. lipolytica* JMY3010 mutant, that overexpress extracellular lipase by the LIP2 gene (encoded the main extracellular lipase activity) cloned under the control of the TEF promoter [3], as also used.

With these different approaches is possible to increase aroma productivity and a greater enhance in ɣ-decalactone production was achieved (up to 7-9 gL^{-1}) through conjugation of a bioprocess optimization and genetic engineering approach.

1. ɣ-decalactone production from castor oil by Yarrowia lipolytica in a bioreactor by operational factors selection. 9th European Congress of Chemical Engineering and 2nd European Congress of Applied Biotechnology. The Hague. The Netherlands, April 21-25, 2013.
2. β-oxidation pathway in the yeast *Yarrowia lipolytica* to increase the production of aroma compounds. Journal of Molecular Catalysis B: Enzymatic. 28:75-79.
3. Une approche de surexpression systématique pour l'identification de nouveaux gènes impliqués dans le métabolisme lipidique chez *Yarrowia lipolytica*. Journées des Microbiologistes de L'INRA. L'Isle-sur-la-Sorgue, November 13 – 15, 2012.

Acknowledgements:The authors acknowledge the European Community fund FEDER through Programa Operacional Factores de Competitividade (COMPETE) and Fundação para a Ciência e a Tecnologia (FCT) (SFRH/BD/63701/2009 PhD) for the financial support provided. We are also grateful to Dr. Jean-Marc Nicaud from the institute MICALIS (INRA-AgroParisTech) for the mutant strains supply.