CO-CULTIVATION OF *Aspergillus nidulans* STRAINS OVER-EXPRESSING HEMICELLULASES FOR ENZYMATIC COCKTAIL PRODUCTION

Damásio ARL[1], Silva TM[2], Segato F[3], Prade RA[3], Jorge JA[2], Polizeli MLTM[2]

[1]Departamento de Bioquímica e Imunologia, FMRP-USP, São Paulo, Brazil

[2]Departamento de Biologia, FFCLRP-USP, São Paulo, Brazil

[3]Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, USA

In today's world, there is an increasing trend towards the use of renewable, cheap and readily available biomass in the production of a wide variety of fine and bulk chemicals in different bioprocesses. Biorefineries utilize the enzymes of microbial cells to convert biomass into target products. The Aspergillus niveus genomic DNA was sequenced by pyrosequencing method and it was identified around 180 target genes encoding to cell wall-degrading enzymes. It was amplified four genes from Aspergillus niveus genomic DNA by PCR reactions encoding for α-L-arabinofuranosidase (ABFase), endo-xylanase (XAN), α-1,5-arabinanase (Arab43) and xyloendoglucanase (XEG). These genes were cloned into pExPYR vector and transformed in A. nidulans through protoplasts and polyethylene DNA fusion. The transformants were selected based on their zeocin-resistance and loss of the uracil and uridine auxotrophy. After that, these strains were cultivated in defined liquid minimum medium added of 5% maltose to pExPYR promoter induction followed by SDS-PAGE protein profile analysis. All described enzymes were over-expressed. Intending the production of an enzymatic cocktail, spores of the four strains over-expressing ABFase, XAN, Arab43 and XEG were inoculated in 5% maltose minimum medium. SDS-PAGE analysis showed a simultaneous production of these four enzymes. The proportion and specific activity of ABFase, XAN, Arab43 and XEG was: 4.23%/5.4 U/mg; 30%/260 U/mg; 40%/67.3 U/mg; 25.3%/33.3 U/mg, respectively. The fungi co-cultivation for simultaneous enzymes expression is an important approach to reduce the enzymatic production cost.

Financial Support: FAPESP and CNPq. Project: INCT Bioetanol.