

# SOCIEDADE BRASILEIRA DE BIOQUÍMICA E BIOLOGIA MOLECULAR



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**PROGRAMA  
E RESUMOS**

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The simultaneous transfer of genes for soybean quality for human nutrition and long juvenile trait is very important to allow the cultivation of the new varieties over a wider environmental range. To evaluate the influence of selection of plants for longer juvenility, on gene frequencies of LOX2, LOX3 and the A<sub>2</sub>A<sub>3</sub>B<sub>3</sub> subunit loci, F<sub>4</sub> seeds from 660 F<sub>3</sub> plants of several crosses between CR<sub>1,3</sub> lines lacking lipoxygenases 2 and 3 and the A<sub>2</sub>A<sub>3</sub>B<sub>3</sub> subunit and the two commercial varieties Paranaíba and Paranaoiana, which present LOX2, LOX3 and the A<sub>2</sub>A<sub>3</sub>B<sub>3</sub> subunit and the long juvenile trait, were analysed. Lipoxygenases were analysed in the seeds by carotene bleaching and the F<sup>2</sup> oxidation tests. The A<sub>2</sub>A<sub>3</sub>B<sub>3</sub> protein subunit was analysed by SDS-PAGE. The statistical analysis of the data showed that there is a significant influence toward the accumulation of LOX3-less genotypes with the selection for longer juvenility. LOX2 and 3 and A<sub>2</sub>A<sub>3</sub>B<sub>3</sub> subunit loci were found to segregate independently.

Financial Support: PADCT/FINEP, CNPq (RHAÉ), NESTLÉ and FAPEMIG.

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LOCALIZATION OF GENES OF AGRONOMICAL INTEREST IN THE RFLP MAP OF COMMON BEANS (*Phaseolus vulgaris*, L.)

M.J. de O. ZIMMERMANN\* and Vallejos, C.E.\*\*.

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\*\* Department of Horticultural Sciences, 1255 Fifield Hall, U. of Florida, Gainesville, FL, U.S.A.

A linkage map of a cross between a black seeded, Mesoamerican common bean line (Jamapa) and a purple mottle seeded, Andean bean line (Calima) was constructed based on 4 isozymes, 3 traits of agronomical interest and 41 restriction fragment length polymorphism loci. An F<sub>2</sub> population of 76 plants, represented by the corresponding 76 F<sub>3</sub> lines was analysed for all these traits.

Linkage groups are corresponding to the linkage groups that had been previously determined in a previous linkage map of common beans constructed from a different cross and using nine seed proteins, nine isozymes 224 RFLPs and a seed and color marker gene P (Vallejos et al., 1992).

Flower color genes that were studied (purple \* white flowers) were located in a different linkage group from that of the P gene. The I gene which conditions hypersensitive response to the necrotic strains of Bean Common Mosaic Virus was located distally in the linkage group D. For that localization, detached leaves of F<sub>2</sub> plants and later the corresponding F<sub>3</sub> lines (16 plants/line) were inoculated with fresh inoculum preparations of strain type NL3.

Vallejos, C.E.; sakiyama, N.S. & Chase, C.D. A molecular marker based linkage map of *Phaseolus vulgaris* L. Genetics, 131: 733-740, 1992.

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BIODEGRADATION OF NATURAL LIGNOCELLULOSICS SUBSTRATES BY *Phanerochaete chrysosporium* AND FEM FUNGI.

Ávila M.H.; Vainstein M.H.; Quirino B.F.; Azevedo M.O.; Felipe M.S.S.

Lab. de Biologia Molecular, Departamento de Biologia Celular, IB, Universidade de Brasília, 70.910-900-Brasília-DF-Brasil.

In our country, there are abundant agricultural and industrial residues such as ball milled straw (BMS) sugar cane bagasse (SCB) and eucalyptus sawdust (ES), which are very important renewable energy sources. We decided to look at the potential use of those residues as substrates for hydrolytic enzymes produced by *Phanerochaete chrysosporium* and a wild-type strain of a fungi, isolated from Amazon, called FEM. Although these two fungi present a similar morphological aspect, they showed variable patterns of amplified bands when submitted to RAPD analysis using 25 different random primers. We have followed, through dot blot RNA hybridization, the expression levels of genes encoding for lignin peroxidases, cellobiohydrolases, xylanases and amylases. The cellulase genes were strongly expressed when the fungi were grown in SCB and BMS and no expression was detected in ES (mineral medium, low nitrogen content). Enzyme profiles showed a high content of exported cellulases, B-glucosidase and xylanase activities only in SCB and BMS. The above results suggest a potential application of both fungi for biotechnological purpose in lignocellulosics bioconversion.

Supported by PADCT/CNPq, FUB, CAPES.

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THE USE OF XYLANASES FROM *Penicillium janthinellum* IN THE BLEACHING OF EUCALYPTUS KRAFT PULP.

A.M.F. Milagres<sup>1</sup>; M.R. Silva<sup>1</sup>; N. Duran<sup>2</sup>; M. Haun<sup>3</sup>.

1-Centro de Biotecnologia - FAENQUIL - Lorena - SP 12600000; 2-Instituto de Química - UNICAMP - Campinas - SP; 3- Instituto de Biologia - UNICAMP - Campinas - SP.

Residual lignin is responsible for the brown color of kraft pulp. Different treatments are applied to those cellulosic fibers to remove this undesirable characteristic. The fibers are treated with chlorine (C), hydrogen peroxide (P), Oxygen (O) and other agents to solubilize or modify the lignin. These process generate wastes that are an environmental hazard due to the formation of chlorinated compounds. Therefore, it is of great relevance the development of an alternative process. One biotechnological answer to this problem is the use of xylanases. Since the hemicellulose seems to be covalently linked to lignin its hydrolyses would facilitate the solubilization/modification of lignin. Therefore, less chemicals would be need to bleach the cellulose. In our laboratory, we have been studying the removal of residual lignin by xylanase from *P. janthinellum*. The enzymatic step were carried out under two combinations of temperature, pH, xylanase concentration and reaction time. Kappa number and viscosity were chosen as the responses to be improved. The optima conditions to maximize the enzymatic treatment was estimated for the the following conditions: temperature 50 °C, initial pH of 5.5, xylanase concentration of 1 U/g of pulp and time of 60 min. Different process for cellulose bleaching have been evaluated combining chemical and enzymatic steps. The enzymatic step has been optimized. In the sequence CED, the pulp pre-treatment with xylanase resulted in 25% reduction of the chlorine needed to achieve the same brightness of a control pulp. Reduction in the requirement for sodium hypochlorite (H) and chlorine dioxide (D) in the delignification of CEHD pulp were 38,6% and 3.6 %, respectively. Short sequences with oxygen and xylanase reduced the kappa number up to 8% with no change in the pulp viscosity.

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PARTIAL PURIFICATION OF LIPASE FROM *Penicillium citrinum*

N.Krieger<sup>3,4</sup>, M.M.Morais<sup>1,2</sup>, M.P.C.Silva<sup>1,2</sup>, J.L.Lima Filho<sup>1,2</sup>, E.H.M.Melo<sup>1,2</sup>,

L.C.B.B.Coelho<sup>1</sup> and G.M.O. Souza<sup>1</sup>.

1. Departamento de Bioquímica/UFPE, Recife-PE
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Microorganisms (germs, fungus and yeast) can produce intracellular lipases or excrete them to the growth medium. The microbial lipases are generally acid proteins with molecular weight range between 20.000 and 60.000 daltons. However, most of them are glycoproteins containing 2 to 15% of carbohydrates, especially D-manose and small amount of D-galactose, D-xylose and D-arabinose.

The industrial application of lipases depends upon the nature and source, conditions of reactions and cost of enzyme production.

Lipase from *Penicillium citrinum* was partially purified using a DEAE-cellulose column (30cm length x 1.5cm width). Lipase solution (2.2ml) was eluted with 50mM citrate-phosphate buffer pH = 5.0, using a flow rate of 15 ml/h. Two protein bands were obtained by SDS-PAGE, electrophoresis of the eluted fractions which contained lipase activity.

The lipase purified will be used in X-ray diffraction study to determine its structure.

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