017 - COMPARISON OF ION EXCHANGE CHROMATOGRAPHY RESINS IN THE MAINTENANCE OF PROTEOLYTIC ACTIVITY OF STEM BROMELAIN FROM PINEAPPLE

ta, H. B.[1], Fernandes, P.M.B.[1], Ventura, J.A[1,2].

1- u leo de Biotecnologia, Universidade Federal do Espirito Santo – UFES; bigtecnol i .ufes@gmail.com ; **2-** Instituto Capixaba de Pesquisa, Assistência Tecnica e Extensão Rural Incaper, Vitória ES; ventura@incaper.es.gov.br.

Bromelain is a group of proteolytic enzymes of great biotechnological interest such as food and pharmaceutical industries. This enz me is present in plants of the Bromeliaceae family, and the pineapple *(Ananas comosus var. comosus)* is the main source for obtaining it. Bromelain has a high commercial value and currently imported into Brazil. Brazil is the world's largest roducer of pineapple but their agricultural residues, rich in this enzyme, haven't been properly exploited. Thus, the development of viable methods for bromelain purification is necessary to reduce costs. This study aimed to compare different ion-exchange chromatography resins in the maintenance of proteolytic activity. The extract obtained from stems of pineapple cv. Vitória was precipitated with ammonium sulfate. The 50-75% fraction was dialyzed and applied on columns of carboxymethylcellulose (CMC), sulfopropilsepharose (SP) and dietilaminoeticelulose (DEAE). Fractions were eluted with different concentrations of acetate buffer, increasing gradually the ionic force, and were collected and screened for the presence of proteins (280 nm) and proteolytic activity (DEPEAU). Fractions with a higher value of proteolytic activity were run on a 15% SDS PAGE. CMC was the resin that preserved proteolytic activity best, with a yield of 89.31%, compared to DEAE (38%) and SP (14%). SDS-PAGE revealed that CMC maintained enzyme integrity without degradation, as was found with SP, and still guaranteed a good purification of the enzyme with a single band (30 kDa), in contrast to the other resins. The results indicate CMC is the best resin for obtaining pre-purified bromelain, and maintaining its high proteolytic activity. Additionally, this shows the possibility of developing a new method of purification, more economic and viable than existing methodologies.

Support: FINEP, CNPq and FAPES

018 - CONSTRUCTION OF METAGENOMIC LIBRARIES WITH LARGE INSERTS OF DNA FROM THE AMAZONIAN SOIL MICROBIOTA AND SCREENING FOR ENZYMES WITH BIOTECHNOLOGICAL POTENTIAL

Jessica C. Bergmann[1], Ohana Y. A. Costa[1], Nídia S. P. L. Ramos[1], Rodrigo S. Furtado[1], Ricardo H. Kruger[3] e Betania F. Quirino[1,2]

1- Universidade Católica de Brasília, Graduate Program in Genomic Sciences and Biotecnology, Brasília, DF, 70.790-160. **2-** Embrapa-Agrienergy, Brasilia, DF, 70.770-901. **3-** Universidade de Brasília, Department of Cell Biology, Enzymology Laboratory, Brasilia, DF, 70910-900

Social, environmental and political reasons are prompting the development of new alternative energy sources. The production of bioethanol from cellulose, the most abundant carbohydrate in nature, is a potential candidate for this purpose. Finding enzymes able to degrade the plant cell wall so that the monosaccharides are released and made available for fermentation in an efficient and economical way has been a challenge. Most enzymes used in industry today come from microorganisms cultured in the lab. Because only 1% of the microbial diversity has been cultured in the lab and Brazil is known for the richness of its flora and fauna, if a similar biodiversity occurs at the microbial level, there is a great unexplored potential for the discovery of novel microbial enzymes. The Amazonian soil has an extensive plant coverage, which makes it a favorable environment for the development of microbial communities. It is known that 1 gram of soil can have the equivalent of 10 million bacteria, an essential component for the stability of the ecosystem. The metagenomic approach can be used to access the genomes and biotechnological potential of uncultivated microorganisms. The purpose of this work was to construct a metagenomic library and search for hydrolytic enzymes that may be used in the production of bioethanol. For this, high molecular weight DNA was directly extracted from the microbial community present in an Amazonian native forest soil collected in the region of Moju, state of Pará. Using Pulse Field Gel Electrophoresis (PFGE) DNA fragments between

40 and 50 kb were selected and excised for library construction in a fosmid vector. The first 3,000 clones obtained have been saved and screened for various activities. Positive clones were identified in screenings for β-glucosidase activity and endoglucanase activities. These positive clones already show the potential of this library although it is still small. The fact that large inserts were used for library construction may allow the identification of enzymes that are part of the same metabolic pathway and perhaps act synergis ically to degrade the plant cell wall. Screenings for other enzymatic activities such as exoglucanase, xylanase, protease and amylase are also being conducted.

Financial Support: CNPq, FAP-DF.

Keywords: Metagenomics, Amazon soil, hydrolytic enzymes, bioethanol.