REUNIÓN CONJUNTA DE SOCIEDADES DE BIOCIENCIAS

EXPRESSION OF HEPATITIS B SURFACE ANTIGEN MAJOR GENOTYPES IN SOUTH AMERICA (F4 AND F1b) IN *PICHIA PASTORIS* AND PURIFICATION FOR *IN VITRO* DIAGNOSIS

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Introduction: Hepatitis B virus (HBV) is one of the main causative agents of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma. Currently, viral strains of HBV are classified into 8 different genotypes designated with letters A-H. F genotype is prevalent in Central and South America.

It has been shown that the immune anti-hepatitis B virus surface antigen (HBs) response to genotype F could be better detected by homologous genotype antigens. As a consequence, we could assess that the use of different antigen genotypes in diagnosis could support a better detection of anti-HBs antibodies against the major genotypes in South America.

In order to achieve this goal, we describe in this study the expression in *Pichia pastoris* and the purification of the HBsAg corresponding to the S region of (sub) genotypes F1b and F4.

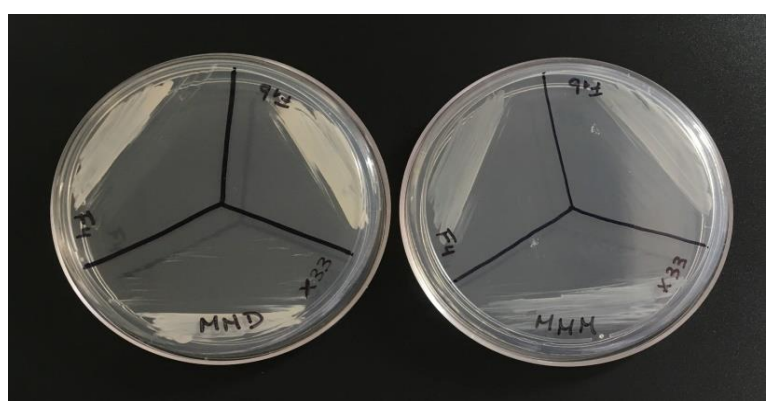

FIGURE 1. Analysis of the phenotype of the clones obtained. Both the F1b and F4 clones grew in the same manner as the x-33 strain on minimal medium dextrose and methanol plates; therefore they constitute a Mut$^+$ phenotype (Methanol Utilization plus).

Materials & Methods

These immunogens were expressed in the methylotrophic yeast *P. pastoris*, as virus-like particles and cultivated to high cell density in 250 ml Erlenmeyer flasks as well as in a 6 L stirred-tank bioreactor. Expression was induced with methanol pulses as the sole carbon source and cultures have been set up for 72 h.

The cells were then lysed by mechanical disruption and the recombinant proteins were purified first by adsorption-desorption on Aerosil™ followed by ultracentrifugation on discontinuous sucrose gradient, dialysis to remove sucrose and finally concentration with 10K AMICON centrifuge tubes.

Purified proteins were analyzed by Coomassie- and silver-stained SDS-PAGE gels and its antigenicity was demonstrated by chemiluminescent microparticle immunoassay (CMIA).

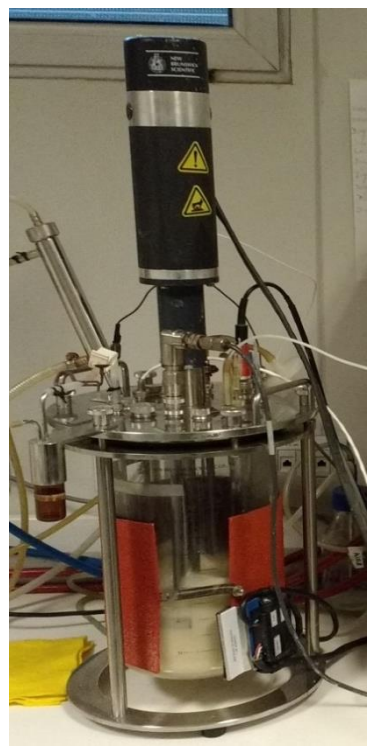

FIGURE 2. 6 L bioreactor of stirred-tank used.

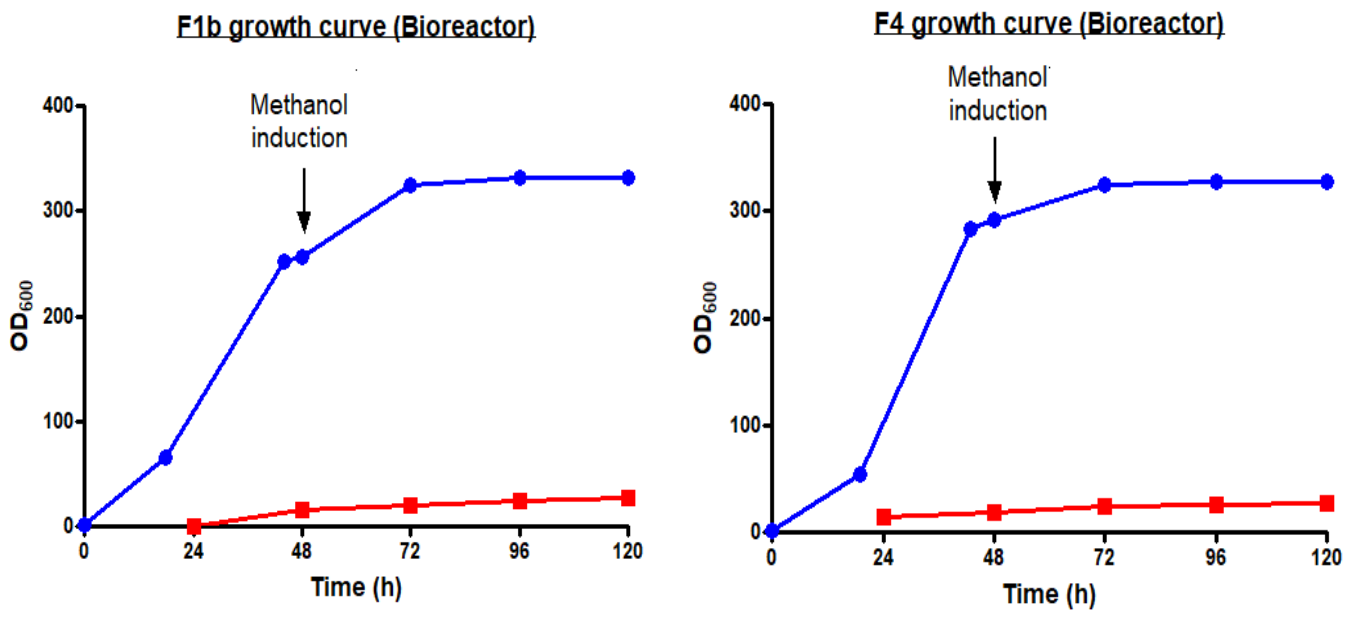

FIGURE 4. Growth curves according to optical density at 600nm in both the Erlenmeyer flasks and the stirred-tank bioreactor of the F1b and F4 subgenotypes. Biomass obtained in bioreactor was about 12-fold higher than in Erlenmeyer flasks for both the F1b and F4 subgenotypes, while 14-fold higher mass of HBsAg was obtained in the bioreactor than in the Erlenmeyer flasks per liter of culture (data not shown).

FIGURE 3. *P. pastoris* cell sediments obtained from the 6 L bioreactor used for the production of HBsAg F genotypes.

RESULTS. All recombinant proteins have been efficiently produced in bioreactor and purified using a short original process.

The antigenicity of the HBsAg from the different (sub) genotypes was measured by CMIA and a reactive value was obtained.

The levels of biomass obtained were approximately 12-fold higher and the mass of HBsAg per liter of culture was 14-fold higher in the bioreactor than in Erlenmeyer flasks.

The purified F4 subgenotype corresponds to the pattern demonstrated by the positive control of pure HBsAg.

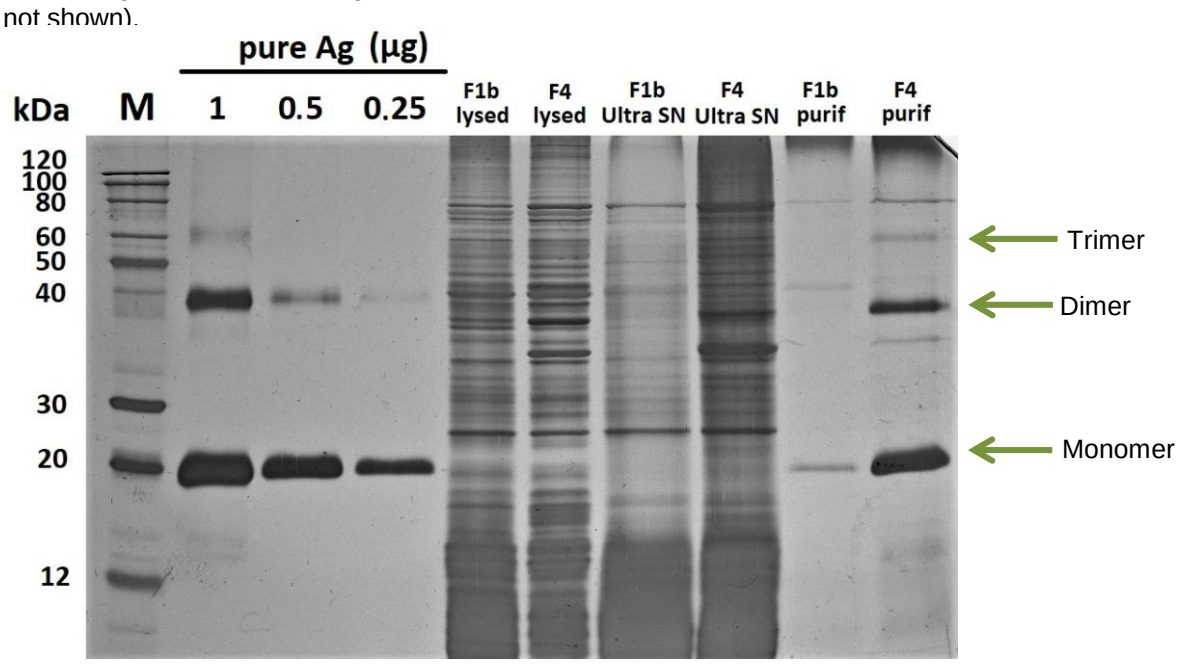

FIGURE 5. Semi quantitation and purification steps. The silver-stained SDS-PAGE gel analysis shows the calibration curve with pure HBsAg (*), starting sample (lysed), the ultracentrifugation supernatant (Ultra SN) and the purified final protein (purif) for both sub genotypes. In the case of purified F4 protein the formation of dimers and trimers in addition to the monomeric recombinant protein it is noted.

Conclusions: The different HBsAg (sub) genotypes expressed in *P. pastoris* are going to be used in a novel immunoassay to assess the usefulness of mixing different genotypes so as to improve the detection efficiency of commercial immunoassays uses for diagnosis.

(*) The authors are very thankful to Pablo Cassará Foundation for giving us the pure HBsAg used as control as a gift.