REUNIÓN CONJUNTA DE SOCIEDADES DE BIOCIENCIAS

NOVEL PURIFICATION METHOD OF VIRUS-LIKE PARTICLES CONTAINING THE HEPATITIS B VIRUS SURFACE ANTIGEN EXPRESSED IN THE METHYLOTROPHIC YEAST *PICHIA PASTORIS*

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Introduction: Virus-like particles (VLPs) are self-assembled systems composed of antigenic proteins, lacking genetic information, that are frequently used for large-scale vaccine production. For instance, currently used hepatitis B vaccines are based on VLPs derived from different eukaryotic systems, such as *Pichia pastoris*. These spherical empty particles are composed of many molecules of the viral S protein (HBsAg). Attempts to purify such immunogen have shown that its antigenicity is sensitive to ammonium precipitation, acidic pH, high ionic strength and PEG; and requires several chromatographic steps.

The aim of this work is to describe a novel purification method that reduces costs, time-consuming steps and makes the process more efficient, simpler and quicker than others.

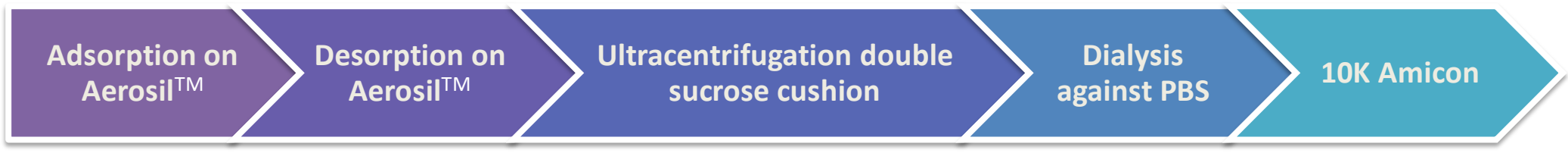
Materials & Methods

The gen corresponding to wild-type HBsAg cloned into the expression vector pPICZa (Life Technologies Corp.) was transformed and intracellularly expressed as VLPs into the *P. pastoris* X-33 strain.

Transformed cells were grown in 250 ml Erlenmeyer flasks at 30°C and were shaken at 250 rpm for 5 days. Induction of recombinant protein expression was performed with 1% methanol final concentration as the sole carbon source.

Cell lysis was performed then by mechanical disruption using glass beads.
The Purification steps were as follows: adsorption-desorption on colloidal silica (Aerosil™) followed by ultracentrifugation on double sucrose cushion, dialysis against PBS buffer and concentration with 10K AMICON centrifuge tubes.
The presence of HBsAg was determined by HPLC mass spectrometry (MS), its antigenicity by chemiluminescent microparticle immunoassay (CMIA) and the assembly of the subviral particles was evidenced by transmission electron microscopy (TEM).

Purification Scheme of wild-type HBsAg



Peptide Sequences	Confidence Level
Peptidic sequences identified by **chymotrypsin** digestion	
(L)NFLGGSPVCL(G)	**High**
(F)LGGSPVCL(G)	**High**
MENITSGF(L)	**High**
(Y)SIVSPF(I)	**High**
(L)GQNSQSPTSNHSPTSCPPICPGY(R)	Medium
Peptide sequences identified by **trypsin** digestion	
(K)YLWEWASVR(F)	Medium

TABLE 1. Peptide sequences obtained by HPLC-MS. The peptide sequences obtained show a percentage coverage of 24.8% with respect to the sequence of the primary structure of HBsAg. Therefore its identity is confirmed.

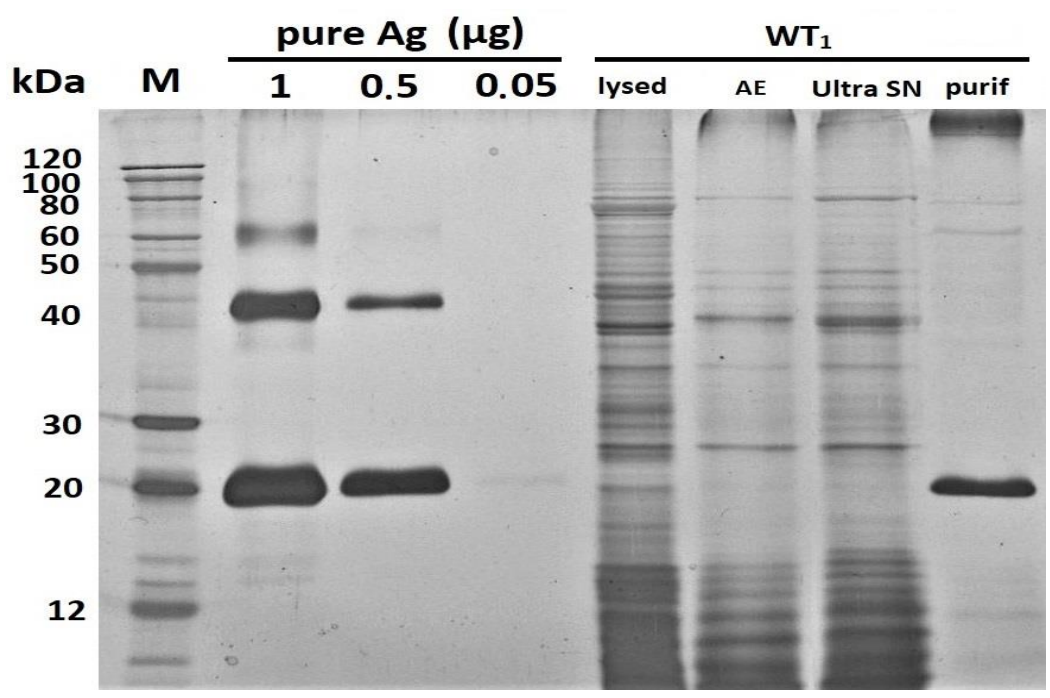
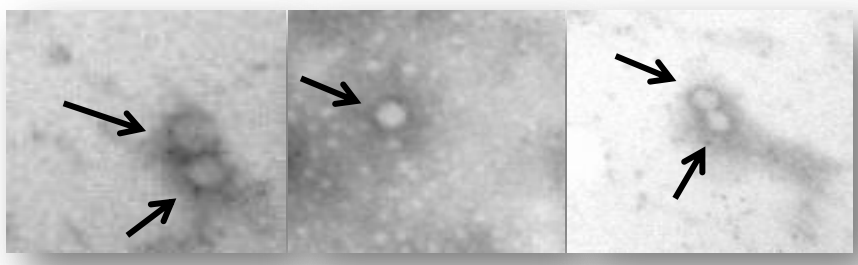


FIGURE 1. Semi quantitation and purification steps. The silver-stained SDS-PAGE gel analysis shows the calibration curve with pure HBsAg (*), starting sample (lysed), the Aerosil desorption step (AE), the ultracentrifugation supernatant (Ultra SN) and the purified final protein (purif) with ~ 90 % of purity, 21 kDa of molecular weight and a final mass of 21.11 µg/ml.

RESULTS: Analysis showed high levels of expression and purity of HBsAg. The antigenicity of the VLPs was maintained according to the positive CMIA results and its identity was corroborated by HPLC-MS. Finally, typical morphological characteristics in size and shape of HBsAg-VLPs were observed by TEM.



N° of VLPs	Average size	Shape
69	(24.3 ± 4.4) nm	spherical

FIGURE 2 and TABLE 2. Morphological analysis of HBsAg-VLPs by Transmission Electron Microscopy (TEM).

Conclusions: the whole purification process described in the present work avoided possible alterations of the morphology and antigenic properties of the HBsAg-VLPs and it was simpler and cheaper than the conventional ones used in industry.

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