RECOMBINANT ENGINEERED GLOBULIN PRODUCTION IN AN INEXPENSIVE CULTURE MEDIUM

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Introduction. The 11S amaranth globulin have a good aminoacid balance, close to the optimum required in the human diet and has remarkable heat stability and emulsifying properties, for these reasons has been proposed as a model to create nutritive and functional foods. This protein is a homohexameric molecule with a molecular mass of 398 kDa and it has two disulfide liked subunits: acidic (32–34 kDa) and basic (22–24 kDa) (1) The acidic subunit has been subjected to modifications by protein engineering, it contains four of the five variable regions of the 11S globulins. This polipeptide was modified by the insertion of VY (4X) antihypertensive peptides in region 3 and IPP in the carboxyl terminus (2).
The purpose of this work was to produce in a reactor the recombinant acidic polypeptide of the 11S globulin of amaranth containing the peptides VY and IPP, using a culture medium containing whey, wherein the lactose acted as an inducer of the expression.

Methods.
Cells of *E. coli* Rosetta (DE3) harboring the plasmid pET-AC-M36His were grown in flask level, using a culture medium containing 3 g / L yeast extract, 10 g / L glycerol, 9.4 and 2.2 g / L of monopotassium phosphate and dipotassium respectively and 990mL of whey. It was determined the level of expression at different times to determine the time of greatest protein accumulation, and subsequently carried out the protein production at reactor level (batch) using agitation of 340 rpm, 37 ° C, 1 vvm, pH was controlled to 6.5 and cell growth was monitored by optical density at 600 nm. Finally, the protein was detected by SDS-PAGE and Western blot and was carried out a densitometry analysis of the images obtained.

Results. The results obtained at flask level showed that the greater accumulation of recombinant protein was at 10 h of cell growth, obtaining 26.6% of total proteins expressed. Figure 1 shows the results at fermenter level, as we can see, the bacteria were able to grow to maximum optical density of 1.6, giving a yield of 596.5 mg / L at 10 h. Figure 2a shows the gel obtained from batch cultivation, here we see a band of approximately 32 kDa, corresponding to the molecular weight of the 11S acidic polypeptide and whose accumulation was gradually increased. From 4 h to 10 h 92% of the interest protein was produced, since there was an increase of 47.2 mg / L to 596.5 mg / L (Figure 2 a and b). Also the yield of protein expressed in the fermenter was 44%, that is 17.4% greater than that obtained in the flask.

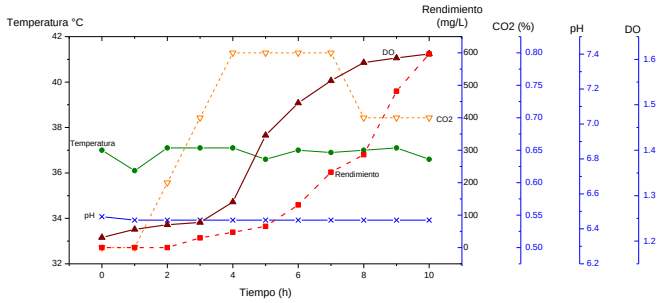

Fig.1 Growth kinetic of E. coli producing the recombinant protein

En la figura 2b también puede observarse el análisis de inmunodetección, en el cual además de confirmar la presencia de proteína recombinante, se puede ver que efectivamente las10 h fue la hora de mayor acumulación de la proteína. Figure 2b also show the immunodetection analysis, it confirm the presence of recombinant protein and can be seen that 10 h was the main accumulation of interest protein.

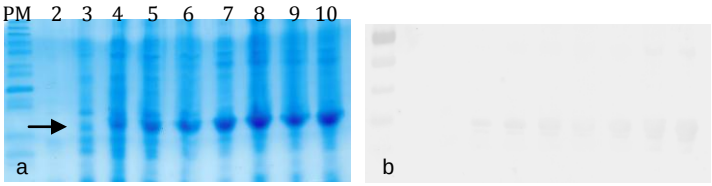

Fig.2 (a) SDS-PAGE analysis and (b) Western blot test.

Conclusions. The whey based medium was effective to produce the expression of the recombinant protein, it is an inexpensive culture medium without the necessity to add an inductor (generally expensive). Yields obtained at reactor level were better than those obtained at flask, 10 h of growth was the best for protein accumulation, the major expressed protein in *E. coli* was the recombinant protein.

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