OVEREXPRESSION OF A MODIFIED AMARANTH PROTEIN AT FERMENTER LEVEL

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Introduction. Hypertension is a cardiovascular disease that affects a large proportion of the population worldwide. Synthetic drugs have been developed for the control of hypertension such as captopril. However, these drugs may cause side effects such as dry cough, increased potassium levels, and skin rashes (1). For therapeutic purposes using protein engineering, further characteristics could be incorporated to protein as biopeptides to enhance nutraceutical properties. Like Castro-Martínez et al. (2) to enhance the nutraceutical properties of amarantin, its acidic subunit has been modified by the insertion of four VY (Val-Tyr) antihypertensive peptides in tandem, into its third variable region. Additionally, the antihypertensive RIPP (Arg-Ile-Pro-Pro) tetrapeptide was inserted in the C-terminal region of the modified amarantin acidic subunit. This study is intended to increase the expression of the modified amarantin acidic subunit at fermenter level.

Methods. *E. coli* Rosetta (DE3) harboring plasmid pET-AC-M3 which codifies the modified amarantin acidic subunit was used in all experiments to express the recombinant protein. Preculture was performed in LB medium supplemented appropriate antibiotics. Preculture was grown overnight at 37°C on a rotary shaker at 200 rpm. The cells were then used to inoculate a 5 L bioreactor. Fermenter cultures were performed 4 L working volume in A7 media (200 g/L potato waste, 12 g/L tryptone, 4 g/L glycerol, 17 mM KH2PO4 and 72 mM K2HPO4). The culture conditions were as follows: inoculum, 2.5% (v/v); pH, 7; agitation, 340 rpm; air flow, 0.1 vvm; temperature protein expression, 30°C. Protein expression was induced with 0.5% lactose (w/v) at OD_{600} = 0.5. 1 mL samples were harvested every hour from 0 up at 24 h by centrifugation to evaluate the expression interest protein. Samples were analyzed by 12% SDS-PAGE (3) and stained with Coomassie brilliant blue G-250. Western blots were performed as described in (2). All protein concentrations were determined using a bicinchoninic acid method using BSA as a protein standard. Gels were scanned and the area and intensity of bands were quantified by densitometric analysis using the ImageJ software.

Results. We observed that the expression of the modified amarantin acidic subunit was accumulated over time and recombinant protein was confirmed by Western blot analysis (data not shown). The cell density for the induction occurred at approximately 4 h of fermentation which coincides with early log phase (Fig. 1). It is possible that the use of suboptimal temperature allowed increased yields of the modified amarantin acidic subunit because it may have reduced the synthesis of heat shock proteases; moreover, in *E. coli* the activity and expression of chaperone proteins are increased at 30°C (4). It was observed that glucose content was minimal at the beginning of the fermentation and it increased in the induction point. That increase in the glucose concentration could be due to the lactose metabolization, this concentration did not repress the recombinant protein expression because of the low concentration. The dissolved oxygen decreased considerably at the induction point and increased at the first harvest (50% of the fermentation volume); increasing the dissolved oxygen could benefit the accumulation of the protein of interest (5). The better productivity was achieved at 4 h of induction with 326 mg/L h. The best yield was at 14h after induction, it was 1835 mg/L of interest protein, better than reported before (2).

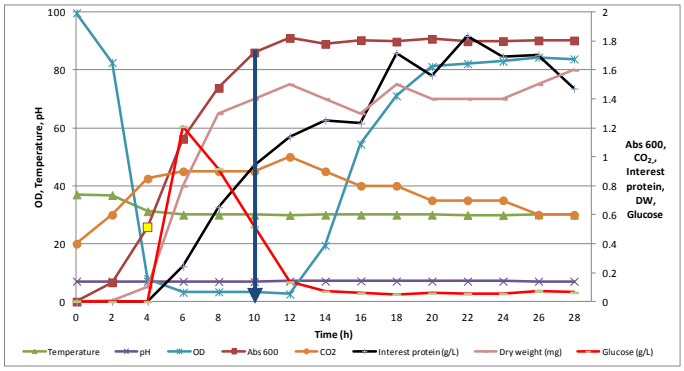

Fig. 1 Growth kinetic of *E. coli* harboring pET-ACM3 and yield of the modified amarantin acidic subunit.

Conclusions. Use of lactose and control of different factors at fermenter level allowed increase the yields of the protein of interest up to 24 h. The best productivity of the recombinant acidic subunit was attained at 4 h of induction and the best yield was1835 mg/L at 14h after induction.

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