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Effect of Three Carbon Sources on the Expression of a Protein with Nutraceutical Properties

Clara Tinixcatl-Herández[1], Edgar Espinosa-Hernández[2], Jocksan Ismael Morales-Camacho[2], Silvia Luna-Suárez[2].

[1]UniversidadInterserrana del Estado de Puebla – Ahuacatlán, México, Department of Biotechnology;
[2]Centro de Investigacion en Biotecnologia Aplicada, CIBA- IPN, Tepetitla, Tlaxcala, Mexico, Department of Biotechnology.

Abstract: The aim of this work was to assess the effect of carbon sources of low cost on the yield of the modified acidic subunit of amarantin, which is the most abundant protein in the seed of amaranth seed, this protein has an excellent aminoacidic balance and has been established as a model for the development of foods with high nutritional quality. Here we express the modified protein in two *E coli* strains, using 3 C sources, the yeast extract was the best source, as we identified the best yield in this medium.

Key words: Carbon source, Expression, Nutraceutical properties, *E. coli*

INTRODUCTION

The predominant storage protein in amaranth seeds is the amarantin, representing about 18.6% of the total grain protein and about 90% of all globulins. This protein is a homo-hexamer with an apparent molecular weight of 300-400 kDa. Contains two subunits, basic and acid, linked by a disulfide bridge[1.]

This protein has a high content of essential amino acids, thus has been established itself as the model for thedevelopment of foods with high nutritional quality and functional properties. Amarantin extracted of seeds has hexameric structure; every monomer is constituted by acidic and basic subunits linked by a disulfide bridge.In recent years there has been a growing interest in certain specific fragments of the dietary protein having biological activity, regulating physiological processes in addition to their nutritional value; as

a result of the digestion of proteins, in addition to free amino acids, bioactive peptides which can pass through the intestinal epithelium and reach peripheral tissues via systemic circulation are released and can perform functions locally specific, systemic and gastrointestinal tract[2].Among the different types of bioactive peptides, those with antihypertensive activity have gained great importance as an increasing incidence of hypertension in the world population, and because cardiovascular disease is the leading cause of death worldwide.

Peptides do possess antihypertensive activity by inhibiting angiotensin converting enzyme (ACE), which is key in the regulation of blood pressure by converting angiotensin I into angiotensin II, a potent vasoconstrictor[2].Through protein engineering, acidic subunit of amaranth has been modified in its third variable region by insertion of the antihypertensive[3], bio-peptide VYVYVYVY. The objective of this research was to evaluate the effect of three carbon sources (of relatively low cost),on the yield of acidic subunit modified, expressed at flask level.

METHODS

E. coli strains BL21–CodonPlus (DE3)-RIL and Rosetta 2 were transformed with plasmid pET-Acid R which codifies to modified protein. The expression was inErlenmeyer flasks containing 50 mL of appropriate medium at 37 ◦C on an orbital shaker (200 rpm). Preculture was performed in 5 mL of LB medium with 100µg mL-1 ampicillin supplemented with 34 µg mL-1chloramphenicol.

Precultures were grown overnight at37°C on a rotary shaker at 200 rpm. The following liquidmedia were used: M1 (12 g L^{-1} yeast extract, 200 g L^{-1} potato waste, 4 g L^{-1} glycerol, 17 mM KH_2PO_4 and 72 mMK_2HPO_4), M2 (12 g L^{-1} commercial yeast, 200 g L^{-1} potato waste, 4 g L^{-1} glycerol, 17 mM KH_2PO_4 and 72 mMK_2HPO_4), and M3 (12 g L^{-1} hydrolyzed yeast, 200 g L^{-1} potato waste, 4 g L^{-1} glycerol, 17 mM KH_2PO_4 and 72 mMK_2HPO_4); 0.5 % (w/v) lactose was used as inducer.

Samples were analyzed by 12% SDS-PAGE and stained with Coomassie brilliant blue G-250. Protein concentration was determined by BCA assay using BSA as a protein standard.

RESULTS

Figure 1 shows that modified protein was expressed with different yields in all media tested.Although in the early hours of induction was observed the presence of recombinant protein (lanes 8 and 9) in both strains, the higher yield was obtained at 10 h of induction.The level of recombinant protein expression was: M1 > M2 > M3 in both strains.

The yields of recombinant protein were higher in M1 media induced with 0.5% (w/v) lactose in both strains but, comparing the expression level at 10h of induction in M1 media (lanes 3 and 6) it is evident that Rosetta 2 strain express higher level of acidic subunit modified than BL21-CodonPlus-RIL, approximately the double yield. The greater accumulation in the M1 broth may be because it contained a better source of nutrients and most bioavailable to *E coli*.

The results obtained are better than reported for amaranth acidic subunit without modification and the modified in the third variable region expressed in Rosseta strain and Origami[3, 4]. DE3.

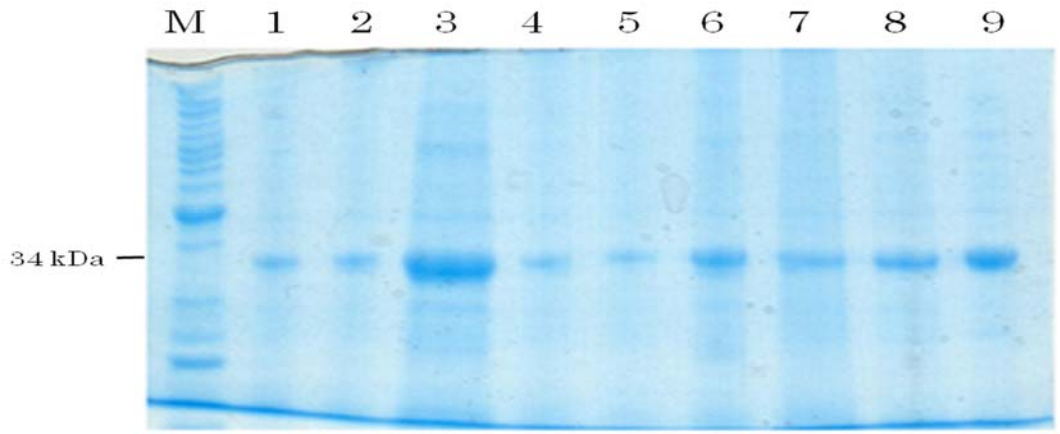

Fig.1: Expression of acidic subunit modified by *E coli* strains. Lanes: M) Molecular weight marker, 1) Rosseta at 10h of induction in M2, 2) Rosseta at 10h ofInduction in M3, 3) Rosseta at 10h of induction in M1, 4) BL21 at10h ofInduction in M2, 5) BL21 at 10 h of induction in M3, 6) BL21 at 10 h ofInduction in M1, 7) Rosseta at 6h of induction in M2, 8) Rosseta at 6h ofInduction in M1, and 9) BL21 at 6h of induction in M1. Approximately 10ug of total protein was loaded into each lane.

CONCLUSIONS

Media M1, with yeast extract, was better broth to express acidic subunit modified with the antihypertensive peptideVYVYVYVY according to yields obtained at 6 h ofinduction with 0.5% of lactose.

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Corresponding author: Silvia Luna-Suárez,

[1]UniversidadInterserrana del Estado de Puebla silvials2004@yahoo.com.mx