Cloning and expression of δ-PalutoxinIT1 fused to the GNA lectin of *G. nivalis* in *E. coli*

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Introduction. Spider toxins such as δ-Palutoxins, μ-agatoxins, δ-atracotoxin among many others, are being recently studied for its neurotoxic activities on voltage dependent ion channels. These peptides have the potential use for the development of insecticidal products as well as the elucidation of ion channel receptor sites and studies of structure-function of these channels. δ-Palutoxins' neurotoxic activity has been tested on previous works and was determined to be insect-selective. Further work is necessary to determine the precise mode of action of these neurotoxins; however a major limiting is the availability of biological material, either the source of the peptides or the purified peptides themselves.

Objective. In this work we propose a novel extracellular expression system for the δ-Palutoxin IT1 based on *psH* signal peptide, the addition of lectin gene and a Histidine tag for prior purification. This strategy may have further application for other peptides or proteins of biotechnological interest.

Methods. *E. coli* BL21 was used for the expression of the fusion protein. The pTrc99 plasmid was used as the expression vector harboring the signal peptide-His Tag-Lectin-Toxin gene fusion (*psH-gna-paluIT1*). The PCR fragments *psH*, *gna* and *paluIT1* were cloned in pTrc99A between the *Nco*I/*Sac*I, *Sac*I/*BamH*I and *BamH*I/*Pst*I restriction sites respectively. The final construction was designated as pTrc*psH-gna-paluIT1*. *E. coli* BL21/pTrc *psH-gna-paluIT1* was cultured in LB medium for 12 at 30 and 37°C Cb (50 μg/ml) and IPTG at 0 and 4 h (0.1 mM). Cell lysis and inclusion bodies purification were carried out using Bugbuster reagent (Novagen). Detection of the recombinant protein on the different culture fractions was carried out by Western blot technique (TransBlot SD Semi Dry, BIORAD) using an anti-HisTag antibody coupled to peroxidase enzyme. Bovine serum albumin (BSA) was used as a negative control.

Results. The total protein concentrations were: supernatant (S.), 1.57 mg/ml; lysed cell extract (C.E.) 2.3 mg/ml per g of cells and 4.96 mg/ml in inclusion bodies (I.B.) per g of cells. The Western blot analysis revealed that the desired fusion protein was successfully expressed, however at 37°C and 0 h induction, most of the total amount of GNA-PaluIT1 was found in I.B. fraction (Fig 1A), thus in the S. and C.E. fractions, no fusion protein was detected with this method under these conditions. S. fraction obtained from cultures carried out at 30°C and IPTG induction after 4 hours was determined to contain the desired fusion protein; important amounts of GNA-PaluIT1 were also found in I.B. fraction (Fig. 1B). Multiple molecular weight bands were detected (arrows), possibly due to incomplete denaturation of protein and the tetramer structure forming nature from GNA.

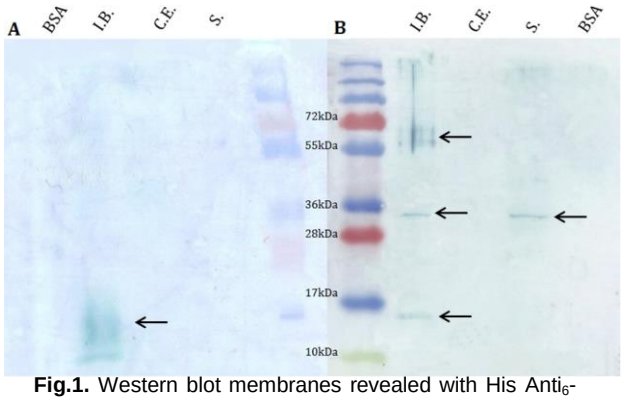

Fig.1. Western blot membranes revealed with His Anti$_6$-peroxidase and peroxidase substrate. A. Fractions obtained from 37°C and 0 h induction cultures. B. Fractions obtained from 30°C and IPTG induction after 4 h.

Conclusions. The system designed in this work allowed the expression and secretion of GNA-PaluIT1 fusion protein; however, the conditions for higher or complete secretion can be optimized.

References.

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