



EXPRESSION OF ALPHA-NEUROTOXIN (MLAT1) OF *MICRURUS LATICOLLARIS* IN *PICHIA PASTORIS*



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Introduction. *Micrurus laticollaris* is a coral snake found in many states of Mexico. People bitten by these snakes present neurotoxic symptoms like flaccid muscle. In some cases it can cause death by respiratory deficiency. These symptoms are caused by alpha and beta neurotoxins which are found in the venom of these snakes.

Currently, our group has found the nucleotide sequence of one alpha neurotoxin (Mlat1) of *M. laticollaris*, and to optimize expression in *Pichia pastoris*, we request the sequence at Genscript Co. with preferential codons for *P. pastoris*, after we cloned that gene in pPICZA, we induced with 1% methanol.

Methods. The *P. pastoris* strain GS115 (his4) and plasmids pPICZA were purchased from Invitrogen (Carlsbad, CA, USA), *Escherichia coli* XL1Blue was used as a host for DNA manipulation, and was grown in LB low salt medium (1% (w/v) tryptone, 0.5% (w/v) yeast extract and 0.5% (w/v) sodium chloride, pH 7.5) containing 25 µg/mL Zeocin. *P. pastoris* was grown in YPD medium (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) dextrose). And YPDS more Zeocin (Sorbitol 1M, 100 µg/mL Zeocin).

P. pastoris strain was transform by electroporation (Invitrogen).

We use BMGY (Buffer phosphate 1M pH 6, Glycerol 1%, Extract of yeast 2%, Peptone of casein 2% and YNB 0.01%) to grow cells at OD₆₀₀ in baffled flask and for expression we use BMMY (Buffer phosphate 1M pH 6,0, Methanol 1%, Extract of yeast 2%, Peptone of casein 2% and YNB 0.01%).

Results

We cloned gene Mlat1 in vector pPICZA and transformed *P. pastoris* GS115, colonies were selected by Zeocin resistance and enzyme restriction. We saw expression by Western blot using His6X monoclonal antibody to recognize the protein (Fig. 1).

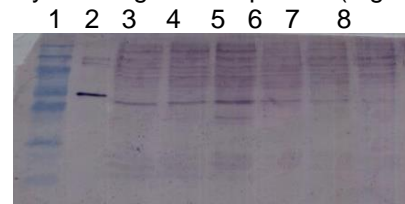


Fig. 1 Western blot, 1. Control His6x, 2. 48h post-induction GS115; 3. 48 h post-induction Gs115-Gal; 4. 48h post-induction GS115-Mlat1; 5. 72h post-induction GS115; 6. 72 h post-induction Gs115-Gal; 7. 72h post-induction GS115-Mlat1;

Conclusions. Mlat1 recombinant can't be expressed in *Pichia pastoris* in flask, oxygen transference is very important to express, because we can't saw expression in positive control GS115-Gal. The use of bioreactor is the best way to express this kind of proteins; also we can monitor oxygen and methanol concentration.

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