EXPRESSION OF PHOSPHOLIPASE A_2 FROM THE VENOM OF *Micrurus laticollaris* IN *Escherichia coli*.

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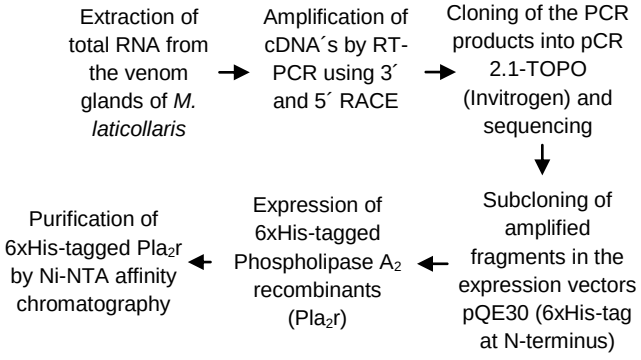
Introduction. There are in Americas, around 70 species of the genus Micrurus snake are found. The bite of Micrurus snakes induces a neurotoxic envenoming, which can lead to peripheral paralysis of respiratory muscles in animals and humans (1). These symptoms are causing by alpha neurotoxins and Phospholipases A_2 (Pla_2) presents in the venom of this snake. Pla_2 are the most abundant component of *Micrurus laticollaris* venom. It has a molecular mass of approximately 12-15 kDa and catalyzes the cleavage 2-*sn*-acyl-ester of glycerophospholipids (2).
The aim of the present study was to generate recombinant Pla_2 antigens for the optimization of neutralizing antivenom. Here, we also describe the cloning of cDNAs coding for Pla_2 isoforms and expression.

Methods.



Results. The RT-PCR yielded bands of the expected size which were cloned and sequenced. In the case of *M. laticollaris*, the deduced amino acid sequences of four distinct sequences were obtained, which we have tentatively called isoforms 1, 2, 3, 4 (Table 1).

6XHis-tagged Pla2 was successfully expressed as inclusion bodies in *E. coli* BL21 (Fig. 1).

Table 1. Identities of reported Pla_2 deduced amino acid sequences.

	Isoform 2	Isoform 3	Isoform 4	Mcor	Mful	NigB	NigA	Naja	Bun
Isoform 1	88	88	94	66	67	65	64	64	53
Isoform 2		88	91	66	67	64	64	66	52
Isoform 3			93	69	68	66	64	68	54
Isoform 4				70	70	69	67	67	55

Isoform 1-4 (*Micrurus laticollaris*), Mcor (*Micrurus corallinus*), Mful (*Micrurus fulvius*), NigB and NigA (*Micrurus nigrocinctus*), Naja (*Naja naja*), Bun (*Bungarus caeruleus*)

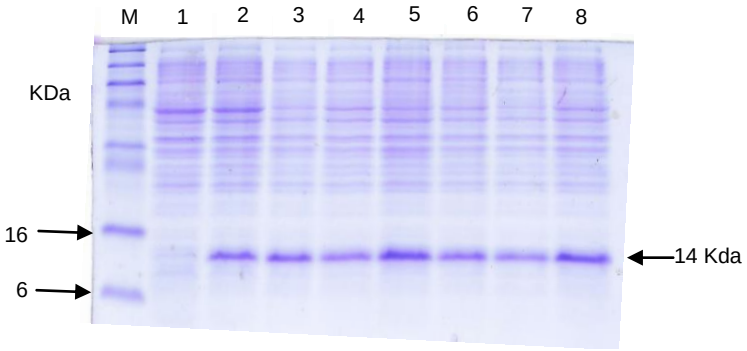


Fig 1. Expression of 6xHis-tagged Pla2 isoform in *Escherichia coli*. M= Molecular weight marker. 1= Total proteins of transformed BL21 without IPTG induction. 2-3= Total proteins of transformed BL21 with IPTG induction isoform 1. 4-5= Total proteins of transformed BL21 with IPTG induction isoform 2. 6-7= Total proteins of transformed BL21 with IPTG induction isoform 3. 8= Total proteins of transformed BL21 with IPTG induction isoform 4.

Conclusions. The PCR products obtained were cloned and sequenced, establishing the complete sequences of the various Pla_2.
The amplified fragments were cloned in *E. coli*-based expression vectors and conditions for expression. The final constructs encoded histidine-tagged polypeptides of 14 kDa.

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References.

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