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Bothrops jararaca venom gland transcriptome: Analysis of the gene expression pattern

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Abstract

Bothrops jararaca is a pit viper responsible for the majority of snake envenoming accidents in Brazil. As an attempt to describe the transcriptional activity of the venom gland, ESTs of a cDNA library constructed from *B. jararaca* venom gland were generated and submitted to bioinformatics analysis. The results showed a clear predominance of transcripts coding for toxins instead of transcripts coding for proteins involved in cellular functions. Among toxins, the most frequent transcripts were from metalloproteinases (52.6%), followed by serine-proteinases (28.5%), C-type lectins (8.3%) and bradykinin-potentiating peptides (BPPs) (6.2%). Results were similar to that obtained from the transcriptome analysis of *B. insularis*, a phylogenetically close sister of *B. jararaca*, though some differences were observed and are pointed out, such as a higher amount of the hypotensive BPPs in *B. insularis* transcriptome (19.7%). Another striking difference observed is that PIII and PII-classes of metalloproteinases are similarly represented in *B. jararaca* in contrast to *B. insularis*, in which a predominance of PIII-class metalloproteinase, which present a more intense hemorrhagic action, is observed. These features may, in part, explain the higher potency of *B. insularis* venom. The results obtained can help in proteome studies, and the clones can be used to directly probe the genetic material from other snake species or to investigate differences in gene expression pattern in response to factors such as diet, aging and geographic localization.
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1. Introduction

Bothrops genus includes more than 30 species and subspecies distributed between Central and South America. Members of this genus are responsible for approximately 90% of venomous snakebite

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accidents in Brazil. *Bothrops jararaca* is the most common species that occurs in the southeast region of Brazil, accounting for the majority of accidents (França and Malaque, 2003; Ribeiro and Jorge, 1997). Clinically, patients bitten by *B. jararaca* usually present edema, systemic bleeding, thrombocytopenia and prolongation of whole blood clotting time (Sano-Martins et al., 1997; Santoro and Sano-Martins, 2004). These symptoms result from the 3 main activities of bothropic venom: proteolytic, with local inflammatory edema at the snakebite site; hemorrhagic, with endothelium damage and systemic bleeding; and procoagulant, leading to the consumption of coagulant factors and disrupting the equilibrium of blood coagulation (Matsui et al., 2000; Varanda and Giannini, 1999). The principal classes of toxins involved in these actions are lectins, prothrombin activating toxins, hemorrhagins, disintegrins and serine-proteinases among others (Markland, 1998; White, 2005).

In this work we generated and analyzed 2318 expressed sequence tags (ESTs) from a cDNA library of the venom gland of *B. jararaca*, aiming to describe its protein contents. Similar transcriptome approach have been conducted with other snakes such as *B. insularis* (Junqueira-de-Azevedo and Ho, 2002), *Bitis gabonica* (Franscischetti et al., 2004) and *B. jararacussu* (Kashima et al., 2004). This kind of study is a good source of information about venom composition, eventually leading to new toxin discovery.

B. jararaca is a close sister taxa of *B. insularis* (Werman, 1992), an endemic snake from Ilha da Queimada Grande, located in São Paulo State (Southeast of Brazil). The geographical isolation and special diet (based exclusively on birds and some invertebrates available on the island) to what *B. insularis* has been submitted for thousands of years may have caused some changes in its venom composition (Daltry et al., 1996). In this work, we adopted the bioinformatic parameters to construct the EST database as close as possible to those used for *B. insularis* transcriptome (Junqueira-de-Azevedo and Ho, 2002), in order to allow comparative studies between these two pit vipers. Therefore, we were able to infer similarities and differences in their venom composition, and correlate it with the potency and clinical symptoms of both venoms.

In our transcriptome analysis, since we have sequenced a larger number of sequence tags, we were able to characterize even not abundant transcripts besides the most frequent ones, providing a set of sequences from a very specialized secretory tissue that can be used for comparative studies and homology searches, and are available at EST section of the NCBI GenBank (http://www.ncbi.nlm.nih.gov/dbEST). Moreover, an EST database can also be used as an auxiliary reference in proteome studies, leading to easier and faster protein identification (Mathesius et al., 2001).

2. Materials and methods

2.1. cDNA library construction and EST technology

The first procedure of an EST project is to obtain mRNA from the tissue of interest, transform it into double-stranded cDNA and clone it into a suitable vector to create a cDNA library. With this intent, poly(A)-rich RNA was prepared from total RNA of the venom gland by oligo(dT)-cellulose chromatography. A directional cDNA library was constructed using a plasmid cloning kit (Superscript plasmid system, Life Technologies Inc.), as described previously (Arocas et al., 1997). The cDNA fragments, representing transcripts for the genes that were expressed at the moment of RNA extraction, were ligated into the pT7T3D EcoRI/NotI/BAP phagemid vector (Pharmacia LKB Biotechnology Inc.) and used to transform *Epicurian coli* KL1 Blue MRF supercompetent cells (Stratagene), producing a library of about 2×105 independent colonies.

An important quality assessment of the library is to determine the average length of the cDNAs that were cloned in the vector and, for this, isolated colonies were randomly chosen and grown in small-scale cultures for plasmid preparation. Plasmid DNA extraction was performed according to Sambrook et al., 1989, and PCR reactions were performed using a pair of primers that flank the multiple cloning site of the vector (T7 upstream and T3 downstream). Amplification conditions were 95 °C for 5 min and 30 cycles of 95 °C for 45 s, 50 °C for 45 s and 72 °C for 4 min, with a final extension step of 72 °C for 5 min. The length of cDNAs was estimated by agarose gel electrophoresis analysis of PCR amplified products.

The next step is the large-scale sequencing of the cDNAs and, for this, aliquots of the plasmid DNA library were used to transform *Escherichia coli* XL1-Blue by electroporation, and plated on Circle Grow medium (Bio 101 Systems, USA) containing

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