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# The sialotranscriptome of the blood-sucking bug *Triatoma brasiliensis* (Hemiptera, Triatominae)

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**Abstract**

*Triatoma brasiliensis* is the most important autochthon vector of *Trypanosoma cruzi* in Brazil, where it is widely distributed in the semiarid areas of the Northeast. In order to advance the knowledge of the salivary biomolecules of Triatominae, a salivary gland cDNA library of *T. brasiliensis* was mass sequenced and analyzed. Polypeptides were sequenced by HPLC/Edman degradation experiments. Then 1712 cDNA sequences were obtained and grouped in 786 clusters. The housekeeping category had 24.4% and 17.8% of the clusters and sequences, respectively. The putatively secreted category contained 47.1% of the clusters and 68.2% of the sequences. Finally, 28.5% of the clusters, containing 14% of all sequences, were classified as unknown. The sialoma of *T. brasiliensis* showed a high amount and great variety of different lipocalins (93.8% of secreted proteins). Remarkably, a great number of serine proteases that were not observed in previous blood-sucking sialotranscriptomes were found. Nine Kazal peptides were identified, among them one with high homology to the tabanid vasodilator vasotab, suggesting that the *Triatoma* vasodilator could be a Kazal protein.


*Keywords:* Saliva; Transcriptome; Hematophagy; Salivary proteins; *Triatoma brasiliensis*

## 1. Introduction

*Triatoma brasiliensis* is the most important autochthonous vector of *Trypanosoma cruzi* in Brazil, where it is widely distributed in the semiarid areas of the Northeast (Costa et al., 2003; Dias et al., 2000; Silveira et al., 1984). This species is able to colonize houses and peridomiciliary areas, and is also widely distributed in sylvatic habitats where it is mainly found among rock piles associated with various species of vertebrates. This increases its importance as a vector, because it can re-colonize domestic habitats after it has been eliminated through insecticide spraying (Alencar, 1987; Diotaiuti et al., 2000).

Blood feeders have evolved a wide set of pharmacologically active molecules to counteract host defense systems (haemostasis, inflammation, immune response) in the feeding site (Andrade et al., 2005; Ribeiro and Francischetti, 2003). Several biomolecules have already been described in triatomine bug saliva, including anticoagulants (Hellmann and Hawkins, 1964, 1965; Pereira et al., 1996; Ribeiro et al., 1998), vasodilators (Ribeiro et al., 1990, 1993; Ribeiro and Nussenzveig, 1993), antihistamines (Ribeiro and Walker, 1994), sialidase (Amino et al., 1998), a sodium channel blocker (Dan et al., 1999), immunosuppressors (Kalvachova et al., 1999), a pore former (Amino et al., 2002), a complement system inhibitor

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(Cavalcante et al., 2003) and inhibitors of platelet aggregation induced by collagen (Noeske-Jungblut et al., 1994; Ribeiro and Garcia, 1981), ADP (Ribeiro and Garcia, 1980; Sarkis et al., 1986), arachidonic acid (Ribeiro and Sarkis, 1982), thrombin (Francischetti et al., 2000; Noeske-Jungblut et al., 1995) or PAF (Golodne et al., 2003). Few of these activities have been reported from *T. brasiliensis*. Sant'Anna et al. (2002), using the suppression subtractive hybridization (SSH) technique, identified six full-length differentially expressed cDNAs from *T. brasiliensis*. Among them, three had their activities inferred by similarity with other sequences in GenBank, but the others had no obvious orthologues.

Until now, only the sialome of *Rhodnius prolixus* has been published (Ribeiro et al., 2004), but the evolutionary diversity among triatomines suggests that extrapolating findings from one group to another should be undertaken with caution. So we can see that there are 138 species in the Triatominae placed in six tribes (Triatomini, Rhodniini, Cavernicoloni, Bolboderini, Alberproseniini, Linschosteini) and 19 genera (Galvão et al., 2003). Schofield (1988) suggested that the Triatominae had different origin from Reduviidae predators that converged to haematophagy habit independently. Several studies showed differences between Rhodniini and Triatomini tribes (Bargues et al., 2000; Catalá, 1997; Dujardin et al., 1999; Garcia and Powell, 1998; Jurberg, 1996; Marcilla et al., 2001; Stothard et al., 1998). Marked differences are also seen between *Rhodnius* and *Triatoma* saliva, in that *Triatoma* lacks nitrophorins, and their apyrase, anticoagulant and vasodilator activities show distinct mechanisms of action (Ribeiro et al., 1998). So a comparative study of a sialome from the Triatomini will be very informative and permit interesting comparisons with that from the Rhodniini already published. Thus, in this study a salivary gland cDNA library of *Triatoma brasiliensis* was mass sequenced and analyzed, and polypeptides were sequenced by HPLC/Edman degradation experiments.

## 2. Materials and methods

### 2.1. Insect rearing

*T. brasiliensis* were captured in Simplício Mendes, Piauí (Northeastern region of Brazil) and reared in the insectary of the Centro de Pesquisas René Rachou—Fiocruz MG, maintained at $28 \pm 2\,^{\circ}C$ and $65 \pm 10\%$ relative humidity. They were kept in cages containing vertical strips of coarse filter paper and fed weekly on chickens.

### 2.2. Salivary gland cDNA library construction

*T. brasiliensis* salivary gland mRNA was isolated from 50 salivary gland pairs from starved adult insects using the Dynabeads mRNA® DIRECT™ kit (DYNAL, Great Neck, NY). The PCR-based cDNA library was constructed using a SMART cDNA library construction kit (BD-Clontech, Palo Alto, CA), according to the manufacturer's instructions. The obtained library was plated by infecting log-phase XL-1 Blue cells (Stratagene, La Jolla, CA, USA). The titer of the cDNA library was $0.492 \times 10^6$ pfu/ml, with a recombination efficiency of 87%.

### 2.3. Sequencing of T. brasiliensis cDNA library

*T. brasiliensis* salivary gland cDNA library was plated to approximately 100 plaques per plate (80 mm Petri dish). The plaques were randomly picked and transferred to 1.5 ml centrifuge microtubes containing 100 µl of distilled water. Five microliters of the phage sample were used as template for a PCR to amplify random cDNAs. The primers TriplEx2-F (5′-CTC CGA GAT CTG GAC GAG C-3′) positioned upstream of the cDNA of interest (5′ end), and TriplEx2-R (5′-TAA TAC GAC TCA CTA TAG GGC-3′) positioned downstream of the cDNA of interest (3′ end) were used for the PCR (94 °C/4 min followed by 35 cycles of 94 °C/1 min, 52.5 °C/1 min and 72 °C/1.1 min, and a final extension of 72 °C/7 min) carried out with the Pht *Taq* DNA polymerase system (Phoneutria, Belo Horizonte, MG, Brazil). Amplified products were visualized by 1.0% agarose gel electrophoresis and cleaned up using the GFX PCR DNA and Gel Band Purification Kit (GE/Amersham Biosciences, Buckinghamshire, UK) or the Wizard® SV Gel and PCR Clean-Up System kit (Promega, Madison, WI, USA). Four microliters of the cleaned PCR product was used as a template for a cycle-sequencing reaction using the DYEnamic ET dye terminator cycle sequencing kit (GE/Amersham Biosciences). The primer Seq. Clontech-F (5′-CTC GGG AAG CGC GCC ATT GTG TTG GT-3′) was used for sequencing. Conditions were 94 °C/1 min, and 35 cycles of 94 °C/30 s, 51 °C/25 s and 60 °C/4 min. After cycle-sequencing the samples, a post-reaction clean-up step, consisted of isopropanol precipitation followed by 70% ethanol wash, was performed. After the supernatant removal, each pellet was dissolved in 6 µL of MegaBACE loading solution and sequenced on a MegaBACE™ 1000 sequencing instrument (GE/Amersham Biosciences).

### 2.4. Chromatography

Approximately 20 µl of saliva from starved adult insects were chromatographed according Ribeiro et al. (2004). Briefly, experiments used 0.24 ml bed volume columns of strong cation (Mono-S) and strong anion (Mono-Q) ion exchangers obtained from Amersham Biosciences (Piscataway, NJ). To elute the proteins of interest, the ion-exchange columns were submitted to gradients of NaCl (0–1 M). For the cation exchange column, the buffer used was 50 mM sodium acetate at pH 5.0 and for the anion exchange, 50 mM Tris-Cl at pH 8.0. Fractions of interest had 40 µl removed and diluted with an equal volume of 20% methanol containing 0.4% tri-fluoroacetic acid (TFA) and were applied to a ProSorb cartridge (Perkin Elmer,