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An insight into the salivary transcriptome and proteome of the soft tick and vector of epizootic bovine abortion, *Ornithodoros coriaceus*☆

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ABSTRACT

The salivary glands of blood-sucking arthropods contain a redundant 'magic potion' that counteracts their vertebrate host's hemostasis, inflammation, and immunity. We here describe the salivary transcriptome and proteomics (sialome) of the soft tick *Ornithodoros coriaceus*. The resulting analysis helps to consolidate the classification of common proteins found in both soft and hard ticks, such as the lipocalins, Kunitz, cystatin, basic tail, hebraein, defensin, TIL domain, metalloprotease, 5′-nucleotidase/apyrase, and phospholipase families, and also to identify protein families uniquely found in the Argasidae, such as the adrenomedullin/CGRP peptides, 7DB, 7 kDa, and the RGD-containing single-Kunitz proteins. Additionally, we found a protein belonging to the cytotoxin protein family that has so far only been identified in hard ticks. Three other unique families common only to the *Ornithodoros* genus were discovered. Edman degradation, 2D and 1D-PAGE of salivary gland homogenates followed by tryptic digestion and HPLC MS/MS of results confirms the presence of several proteins. These results indicate that each genus of hematophagous arthropods studied to date evolved unique protein families that assist blood feeding, thus characterizing potentially new pharmacologically active components or antimicrobial agents.

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1. Introduction

Soft ticks (Argasidae) feed exclusively on blood, but unlike the related hard ticks (Ixodidae) that feed for several days, soft tick meals last at most 1 h. Argasidae also feed repeatedly as adults, laying smaller egg batches per meal than hard ticks. In their adaptation to blood feeding, arthropods have evolved a sophisticated cocktail of salivary components that disarm

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their host's hemostatic system (consisting of platelet aggregation, vasoconstriction, and blood clotting), as well as opposing inflammatory and immunologic reactions [1]. Salivary transcriptomes (sialotranscriptomes; "sialo"; Greek: saliva, spittle, foam from the mouth; the salivary glands) of several hard tick species [2–7], as well as from the soft ticks *Ornithodoros parkeri* and *Argas monolakensis* [8–10] have been characterized. These studies indicate that the transcriptome repertoire of hard ticks have a larger complexity than those of soft ticks, probably deriving from the increased needs of hard ticks to counteract their hosts' immune, inflammatory and angiogenic responses that occur on prolonged feeding. On the other hand, these same transcriptomes indicate a large common expansion of genes coding for several protein families, including metalloproteases, cysteine-rich proteins similar to metalloprotease domains (the ixostatins and ixodegrins), lipocalins, Kunitz-domain-containing proteins, RGD-containing peptides, defensins, and many novel protein families that may include antimicrobial proteins. Argasidae-specific families have also been identified.

In the present study, we enlarge the dataset of Argasidae salivary gland transcripts by exploring that of *Ornithodoros coriaceus*, a vector of epizootic bovine abortion in the western U.S. [11–13]. The main protein families found in argasid and ixodid sialotranscriptomes display a low degree of similarity, which supports the idea that salivary proteins are evolving at a very fast rate compared to that of housekeeping proteins. The current dataset also contributes to expanding the tick sialotranscriptome landscape and aids in the eventual mapping of the evolutionary pathways leading to the diverse protein family expansions observed today.

2. Materials and methods

2.1. Ticks

Tick salivary gland extracts were prepared by collecting glands from *O. coriaceus* adult ticks. Glands were dissected by first bisecting the tick and then teasing the salivary glands away from the other internal organs and the tick exoskeleton. Glands were rinsed by immersion in PBS and added to 10 μl of distilled water (for 1D or 2D gel electrophoresis), or RNA later overnight at 4 °C (for mRNA extraction), and stored frozen at −75 °C until further analysis.

2.2. Chemicals

Standard laboratory chemicals were purchased from Sigma Chemicals (St. Louis, MO) if not specified otherwise. Formic acid and trifluoroacetic acid (TFA) were obtained from Fluka (Milwaukee, WI). Trypsin was purchased from Promega (Madison, WI). HPLC-grade acetonitrile was from EM Science (Darmstadt, Germany), and water was purified by a Barnstead Nanopure system (Dubuque, IA).

2.3. Salivary gland isolation and library construction

O. coriaceus salivary gland mRNA from 5 pairs of glands was isolated using the Micro-FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). The PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech, Palo Alto, CA). This system utilizes oligoribonucleotide (SMART IV) to attach an identical sequence at the 5′ end of each reverse-transcribed cDNA strand. This sequence is then utilized in subsequent PCR reactions and restriction digests.

First-strand synthesis was carried out using PowerScript reverse transcriptase at 42 °C for 1 h in the presence of the SMART IV and CDS III (3′) primers. Second-strand synthesis was performed by a long-distance (LD) PCR-based protocol using Advantage™ Taq Polymerase (Clontech) mix in the presence of the 5′ PCR primer and the CDS III (3′) primer. The cDNA synthesis procedure resulted in the creation of SfiI A and B restriction enzyme sites at the ends of the PCR products that are used for cloning into the phage vector. The PCR conditions were: 95 °C for 20 s; 24 cycles of 95 °C for 5 s, 68 °C for 6 min. A small portion of the cDNA obtained by PCR was analyzed on a 1.1% agarose gel to check for the quality and range of cDNA synthesised. Double-stranded cDNA was immediately treated with proteinase K (0.8 μg/ml) at 45 °C for 20 min, and the enzyme was removed by ultrafiltration though a Microcon (Amicon Inc., Beverly, CA) YM-100 centrifugal filter device. The cleaned, double-stranded cDNA was then digested with SfiI at 50 °C for 2 h, followed by size fractionation on a ChromaSpin-400 column (Clontech). The profile of the fractions was checked on a 1.1% agarose gel, and fractions containing cDNAs of more than 400 bp were pooled and concentrated using a Microcon YM-100.

The cDNA mixture was ligated into the λ TriplEx2 vector (Clontech), and the resulting ligation mixture was packaged using the GigaPack® III Plus packaging extract (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The packaged library was plated by infecting log-phase XL1-Blue *Escherichia coli* cells (Clontech). The percentage of recombinant clones was determined by performing a blue-white selection screening on LB/$MgSO_4$ plates containing X-gal/IPTG. Recombinants were also determined by PCR, using vector primers (5′ λ TriplEx2 and 3′ λ TriplEx2 sequencing primers) flanking the inserted cDNA and visualizing the products on a 1.1% agarose/EtBr gel.

2.4. Sequencing of the *O. coriaceus* cDNA library

The *O. coriaceus* salivary gland cDNA library was plated on LB/$MgSO_4$ plates containing X-gal/IPTG to an average of 250 plaques/150-mm Petri plate. Recombinant (white) plaques were randomly selected and transferred to 96-well MICROTEST™ U-bottom plates (BD BioSciences, Franklin Lakes, NJ), containing 100 μl of SM buffer (0.1 M NaCl; 0.01 M $MgSO_4$; 7 H_2O; 0.035 M Tris–HCl (pH 7.5); 0.01% gelatin) per well. The plates were covered and placed on a gyrating shaker for 30 min at room temperature. The phage suspension was either immediately used for PCR or stored at 4 °C for future use.

To amplify the cDNA using a PCR reaction, 4 μl of the phage sample was used as a template. The primers were sequences from the λ TriplEx2 vector and named pTEx2 5seq (5′-TCC GAG ATC TGG ACG AGC-3′) and pTEx2 3LD (5′-ATA CGA CTC ACT ATA GGG CGA ATT GGC-3′), positioned at the 5′ end and the 3′ end of the cDNA insert, respectively. The reaction was carried out in 96-