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Proteome analysis of abundantly expressed proteins from unfed larvae of the cattle tick, *Boophilus microplus*

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Abstract

Protein expression in unfed larvae of the cattle tick, *Boophilus microplus*, was characterized using gel electrophoresis and mass spectrometry in an effort to assemble a database of proteins produced at this stage of development. Soluble and insoluble proteins were extracted and resolved by two-dimensional (2D) gel electrophoresis. Twenty abundantly expressed larval proteins were selected for peptide mass mapping and for peptide sequencing by matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) and quadrupole time-of-flight (Q-ToF) tandem mass spectrometry (MS), respectively. Only one protein, tropomyosin, was unequivocally identified from its peptide mass map. Ten proteins were assigned putative identities based on BLAST searching of heterologous databases with peptide sequences. These included a cytoskeletal protein (troponin I), multiple cuticular proteins, a glycine-rich salivary gland-associated protein and proteins with a presumed housekeeping role (arginine kinase, a high-mobility group protein and a small heat shock protein). Eight additional proteins were identified by searching translated open reading frames of a *B. microplus* EST database (unpublished): putative fatty-acid binding protein, thioredoxin, glycine-rich salivary gland protein and additional cuticular proteins. One remaining protein was not identifiable, suggesting it may be a novel molecule. The ongoing assembly of this database contributes to our understanding of proteins expressed by the tick and provides a resource that can be mined for molecules that play a role in tick-host interactions.
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1. Introduction

Rapid advances in genomic and proteomic technologies have stimulated a revolution in molecular entomology, as reflected in the increasing number of publications on arthropods utilizing such approaches. These include studies on the fruit fly, *Drosophila melanogaster* (Vierstraete et al., 2003), as well as vectors of disease, including tsetse flies that spread African trypanosomes (Haddow et al., 2002; Haines et al., 2002), *Ixodes scapularis*, the main vector of Lyme disease (Valenzuela et al., 2002) and *Rhodnius prolixus*, the vector of Chagas disease (Ribeiro et al., 2004).

The cattle tick, *Boophilus microplus*, is an ectoparasitic arthropod that is a vector of *Babesia spp* and *Anaplasma marginale*, the causative agents of bovine babesiosis and anaplasmosis, respectively. *B. microplus* was essentially eradicated from the United States in 1943 (Graham and Hourrigan, 1977). However, *B. microplus* is prevalent in Mexico and hundreds of thousands of cattle are imported annually from Mexico into the United States (Bram et al., 2002). A quarantine zone is maintained along the Texas-Mexico border to prevent the re-entry of the tick into the US, and a stringent quarantine program at border import facilities involves the physical inspection and dipping of cattle in vats of an acaricide

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prior to their importation. The prolonged and intensive use of acaricides for the control of the tick population has contributed to the development of acaricide resistant populations of ticks (Ortiz Estrada et al., 1995; Villarino et al., 2002; Li et al., 2003), necessitating the design of novel control strategies.

As *B. microplus* is a one-host tick, it must cope with the pressures of the immune response resulting from the feeding of all lifestages on a single host. The identification of proteins expressed at the different lifestages of the tick will provide a resource that could be mined for biologically important molecules for the development of novel control strategies. The work presented here describes the identification of 19 proteins that are abundantly expressed during the larval stage of *B. microplus* development. It is our intent that this information will be used in the assembly of a two-dimensional (2D) database of expressed larval proteins, which will extend our understanding of the repertoire of molecules produced by the tick and aid in the identification of proteins to which the host elicits an immune response at this stage.

2. Materials and methods

2.1. Tick strains

The *B. microplus* organophosphate (coumaphos) resistant strain, San Roman (SR), was collected in 1994 from a ranch located in Champoton, Campeche, Mexico. The strain was maintained at the Cattle Fever Tick Research Laboratory (CFTRL) in Mission, TX, as described by Davey et al. (1980). Unfed larvae between 12 and 16 days old were collected and were immediately frozen at −80 °C until subsequent use.

2.2. Sample preparation

Soluble and insoluble proteins were isolated from larvae using the ReadyPrep™ Sequential Extraction Kit (BioRad, Hercules, CA). Tick larvae (0.6 g) were ground to a powder in BioRad Reagent 1 (40 mM Tris base) using a liquid nitrogen cooled mortar and pestle. The sample was further homogenized using three 10 s bursts with a Polytron, treated with FOCUS-Nuclease™ (Genotech, St. Louis, MO) and a protease inhibitor cocktail (FOCUS-Protease Arrest™; Genotech) for 1 h at room temperature and subsequently ultracentrifuged at 210,000 × *g* for 4 h at 4 °C. Supernatant, consisting of soluble cytosolic proteins, were stored in 300 μl aliquots at −20 °C. Urea-soluble, putative membrane or membrane-associated proteins were further extracted by resuspending the remaining pellet in BioRad Reagent 2, a buffer consisting of 8 M urea, 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 2 mM tributylphosphine (TBP), 40 mM Tris, and 0.2% (w/v) Bio-Lyte™ pH 3–10 ampholytes (BioRad). Highly insoluble proteins were removed from this mixture by ultracentrifugation at 210,000 × *g* for 2 h at 25 °C, and the membrane protein-enriched supernatants were collected in 300 μl aliquots and stored at −20 °C.

Protein aliquots were extracted with an equal volume of water-saturated phenol, and proteins were precipitated from the phenol phase using 0.1 M ammonium acetate in methanol overnight at −20 °C, as described by Hurkman and Tanaka (1986). The precipitate was washed three times with 0.1 M ammonium acetate in methanol and once with acetone prior to solubilization in BioRad Reagent 2.

The protein concentration of each sample was determined using the modified BioRad Protein Assay System, based on the Bradford dye-binding procedure (Bradford, 1976). The supernatants were analyzed by 2D gel electrophoresis.

2.3. Two-dimensional gel electrophoresis. First dimension: isoelectric focusing (IEF)

A 300 μl sample containing 400 μg of protein diluted in BioRad Reagent 2 was used to rehydrate Ready-Strip™ Immobilized pH Gradient (IPG) strips (17 cm, linear pH 3–6, 5–8 and 7–10; BioRad) for at least 18 h at room temperature. Additionally, a 125 μl sample containing 50 μg of protein diluted in the same buffer was utilized for the rehydration of 7 cm IPG strips for each of the various pH ranges. IEF was performed using the PROTEAN® IEF Cell (BioRad) according to the manufacturer's protocol. Focused strips were stored at −20 °C.

2.4. Two-dimensional gel electrophoresis. Second dimension: gradient gel electrophoresis

The focused IPG strips were equilibrated for 15 min at room temperature in a buffer comprised of 6 M urea (w/v), 2% SDS (w/v), 0.05 M Tris, pH 8.8, 20% glycerol (v/v), and 2% DTT (w/v). A second equilibration of 15 min was conducted in the same buffer with 2.5% iodoacetamide (w/v) instead of 2% DTT. Equilibrated strips (17 cm) were transferred to the IPG well of a PROTEAN®II ReadyGel®, (8–16% acrylamide gradient; BioRad) and the second-dimensional electrophoresis was conducted in Tris/Glycine/SDS buffer (BioRad) at 24 mA/gel on a PROTEAN® II xi apparatus (BioRad). The shorter equilibrated strips (7 cm) were transferred to the IPG well of a ReadyGel®, (8–16% acrylamide minigel; BioRad) and electrophoresed in the same buffer at 170 V for 75 min on a MiniProtean®II apparatus (BioRad) at 4 °C.

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