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Insect Biochemistry and Molecular Biology 35 (2005) 585–595
Insect Biochemistry and Molecular Biology
www.elsevier.com/locate/ibmb

BmiGI: A database of cDNAs expressed in *Boophilus microplus*, the tropical/southern cattle tick

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Received 19 November 2004; accepted 26 January 2005

Abstract

We used an expressed sequence tag approach to initiate a study of the genome of the southern cattle tick, *Boophilus microplus*. A normalized cDNA library was synthesized from pooled RNA purified from tick larvae which had been subjected to different treatments, including acaricide exposure, heat shock, cold shock, host odor, and infection with *Babesia bovis*. For the acaricide exposure experiments, we used several strains of ticks, which varied in their levels of susceptibility to pyrethroid, organophosphate and amitraz. We also included RNA purified from samples of eggs, nymphs and adult ticks and dissected tick organs. Plasmid DNA was prepared from 11,520 cDNA clones and both 5′ and 3′ sequencing performed on each clone. The sequence data was used to search public protein databases and a *B. microplus* gene index was constructed, consisting of 8270 unique sequences whose associated putative functional assignments, when available, can be viewed at the TIGR website (http://www.tigr.org/tdb/tgi). A number of novel sequences were identified which possessed significant sequence similarity to genes, which might be involved in resistance to acaricides.
Published by Elsevier Ltd.

Keywords: *Boophilus microplus*; Acaricide resistance; Acetylcholinesterase; Expressed sequence tags; Glutathione *S*-transferase; Cytochrome P450

1. Introduction

Boophilus ticks are present in many parts of the world. Although recently reclassified to the genus *Rhipicephalus*, for the purposes of biological clarity, we maintain use of the previous genus assignment (Murrell and Barker, 2003). *Boophilus microplus*, the tropical or southern cattle tick, is the most widely distributed *Boophilus* species and the most economically important. Originally from Asia, this one-host tick species has colonized most of the world's tropical and sub-tropical countries, with favorable ecological conditions facilitating its spread to much of Africa, Central and South America and Australia (McCosker, 1979). *B. microplus* and the blue tick, *B. decoloratus*, are the two most important *Boophilus* vectors of pathogens that affect the global cattle and small ruminant population. These ticks transmit protozoan (*Babesia bovis* and *B. bigemina*) and prokaryotic (*Anaplasma marginale*) organisms, which cause babesiosis and anaplasmosis, respectively. The tick-disease complex of *Boophilus* spp.—*Babesia* spp.—*A. marginale* is probably the most important agriculturally related complex world-wide (de Castro, 1997), leading to severe losses in milk and beef production and restriction in traffic of livestock. The ticks also directly add to this economic burden as they cause hide damage, toxicosis and stress.

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0965-1748/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.ibmb.2005.01.020

The major method of tick and tick-borne disease control is the application of acaricides to kill the tick vector. However, *B. microplus* has developed acaricide resistance, and, in many countries, the resistance phenotype extends to several chemical groups such as arsenicals, organochlorines, organophosphates, amidines and pyrethroids (Angus, 1996; Kemp et al., 1998; Miller et al., 1999). The loss of the ability to control *B. microplus* has severe economic consequences for cattle producers. For example, in Australia, the tick has acquired resistance to pyrethroid, organophosphate and amitraz acaricides, resulting in reported annual losses of $100 million/year (Angus, 1996). Livestock production losses would likely be considerably higher were it not for use of a combined attenuated live vaccine against babesiosis and anaplasmosis along with an anti-tick vaccine as part of an integrated tick and disease control strategy.

The inadvertent importation of tick-infested cattle into the United States around the early part of the 19th century resulted in the rapid establishment of *B. microplus* and *Boophilus annulatus* ticks in 14 southern states and southern California and the concurrent transmission of bovine babesiosis (Graham and Hourrigan, 1977). The US cattle industry's annual losses attributable to *Boophilus* ticks were estimated in 1906 to be $130,500,000 and led to a federal-state *Boophilus* eradication program initiated in 1906. The program was essentially completed by 1943 but the ticks were not completely eliminated from Florida until 1960 and outbreaks still occur in Texas, generally confined along the US-Mexico border (Graham and Hourrigan, 1977).

Owing to the widespread prevalence of pathogen-infected *Boophilus* ticks in Mexico, the eradication status is maintained using a 500-mile long and 1/4 to 10 miles wide "buffer zone" along the US-Mexico border with mandatory acaricide treatment of livestock prior to importation into the US (George et al., 2002; Bram et al., 2002). The development in Mexico (and throughout Central and South America) of *B. microplus* populations resistant to multiple acaricides, particularly acaricides approved for use in the border "buffer zone" treatment vats, is a major risk factor for re-introduction and re-establishment of this vector to the US.

The availability of organism-specific whole genome sequence data has revolutionized approaches to the study of biological systems, leading to novel insights and opportunities for solving biological problems that affect human health, agriculture and the environment. Because of the high cost of whole genome sequencing projects, expressed sequence tag (EST) projects, single pass sequencing of cDNA ends, are likely to remain a major source of organism-specific DNA sequence data for many eukaryotic species. Such gene discovery projects are likely to benefit the tick and tick-borne disease research community as they form the framework for enhancing our understanding of tick biology and molecular mechanisms, which contribute to parasitism, acaricide resistance, and trans-stadial and trans-ovarial pathogen transmission. Such insights may be translated into novel tick and tick-borne disease control options through the identification of biological targets for developing novel chemotherapeutic chemistries and vaccines to effect tick control (Hill and Gutierrez, 2000).

Prior to this work, GenBank contained a total of 752 accessions for *B. microplus*, with less than 20% of these sequences representing protein coding genes. A previous project reported on the generation of 234 unique ESTs from whole *B. microplus* larvae (Crampton et al., 1998). In this paper, we report on the construction of a gene index called BmiGI from 20,417 ESTs derived from a normalized cDNA library and assignment of potential function to the sequences by use of an auto-annotation pipeline. This data represents a two-fold increase in GenBank sequence information for organisms in the sub-order Ixodida and will allow development of high throughput techniques and hypothesis-based research in the study of tick biology and vector-pathogen interactions. In light of the threat posed by acaricide resistant ticks to the *Boophilus*-free status of the US, BmiGI was analyzed to identify genes which might be involved in acaricide resistance, including genes encoding the target site of action of specific pesticides and enzymes involved in detoxification or sequestration of pesticides. Because of the critical role organophosphates play in the "buffer zone" treatment vat program, we focused on genes encoding proteins with established roles in promoting organophosphate resistance. Acetylcholinesterase (AChE) is the target of organophosphates and resistance-associated mutations have been noted in AChE coding regions from *Drosophila melanogaster* (Mutero et al., 1994), *Musca domestica* (Walsh et al., 2001) and *Aedes aegypti* (Vaughan et al., 1997). A number of sequences in BmiGI were identified which encode proteins with significant amino acid similarity to AChE. Additionally, sequences were found which encoded proteins with similarity to carboxylesterase, glutathione *S*-transferase, and cytochrome P450, all of which are large gene families having members implicated in pesticide resistance mechanisms through detoxification or sequestration (Jamroz et al., 2000; Scott, 1999; Wei et al., 2001).

2. Material and methods

2.1. Tick rearing

Ticks were reared at the USDA-ARS Cattle Fever Tick Research Laboratory in Mission, TX, as described by Davey et al. (1980). *B. microplus* larvae infected with *B. bovis* were obtained from Dr. Don Knowles (USDA-ARS Animal Disease Research Unit, Pullman,