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Expressed sequence tags from the red imported fire ant, *Solenopsis invicta*: Annotation and utilization for discovery of viruses

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Abstract

An expression library was created and 2304 clones sequenced from a monogyne colony of *Solenopsis invicta*. The primary intention of the project was to utilize homologous gene identification to facilitate discovery of viruses infecting this ant pest that could potentially be used in pest management. Additional genes were identified from the ant host and associated pathogens that serve as an important resource for studying these organisms. After assembly and removal of mitochondrial and poor quality sequences, 1054 unique sequences were yielded and deposited into the GenBank database under Accession Nos. EH412746 through EH413799. At least nine expressed sequence tags (ESTs) were identified as possessing microsatellite motifs and 15 ESTs exhibited significant homology with microsporidian genes. These sequences most likely originated from *Thelohania solenopsae*, a well-characterized microsporidian that infects *S. invicta*. Six ESTs exhibited significant homology with single-stranded RNA viruses (3B4, 3F6, 11F1, 12G12, 14D5, and 24C10). Subsequent analysis of these putative viral ESTs revealed that 3B4 was most likely a ribosomal gene of *S. invicta*, 11F1 was a single-stranded RNA (ssRNA) virus contaminant introduced into the colony from the cricket food source, 12G12 appeared to be a plant-infecting tenuivirus also introduced into the colony as a field contaminant, and 3F6, 14D5, and 24C10 were all from a unique ssRNA virus found to infect *S. invicta*. The sequencing project illustrates the utility of this method for discovery of viruses and pathogens that may otherwise go undiscovered. Published by Elsevier Inc.

Keywords: *Solenopsis invicta*; Expressed sequence tag; Expression library; Dicistroviridae; Microsporidia

1. Introduction

The black imported fire ant, *Solenopsis richteri*, and red imported fire ant, *Solenopsis invicta*, were introduced into the United States around 1918 (Creighton, 1950) and between 1933 and 1945 (Lennartz, 1973), into Mobile, Alabama, respectively. The black imported fire ant is confined currently to a small area in northern Alabama and Mississippi, and western Tennessee. The more successful red imported fire ant has spread to 128 million hectares from Virginia south to Florida and west to California (Williams et al., 2001) with mound densities as high as 200 per hectare (Macom and Porter, 1996). The red imported fire ant is highly aggressive and considered the dominant arthropod in infested areas (Vinson and Greenberg, 1986). The estimated damage caused by these ants exceeds 1 billion dollars annually in the U.S. (Thompson et al., 1995).

Although chemical insecticides are highly effective at controlling fire ants, they provide only temporary population suppression (Williams et al., 2001). Infested areas cleared of fire ants can only be maintained fire ant free with continuous insecticide use; population levels invariably rebound when insecticide treatment ceases (Tschinkel,

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2006). Because fire ants are so ubiquitous within the infested region of the U.S., insecticide control is impractical. It is generally accepted that the most tenable approach to achieve sustainable control of imported fire ants is the establishment of biological and microbial organisms specific to these ant species (Porter et al., 1997; Tschinkel, 2006). Evidence supporting this notion includes several studies comparing *S. invicta* population characteristics in the United States and in its native range, South America, where fire ants are not considered significant pests. In the U.S., fire ant populations are significantly greater (5-fold), found in higher densities (6-fold), possess larger mound volumes (2-fold), and comprise a larger fraction of the ant community (7.5-fold) than in South America (Porter et al., 1992, 1997). Furthermore, over 30 fire ant natural enemies have been identified in South America—nearly all of which are absent among U.S. populations (Jouvenaz et al., 1977, 1981; Jouvenaz, 1983; Wojcik et al., 1987; Porter et al., 1997; Williams et al., 2003). Thus, as Porter et al. (1997) suggest, *S. invicta* and *S. richteri* likely escaped from their natural enemies in South America when they were introduced into the U.S. Therefore, increasing the number and efficacy of fire ant-specific microbial and biological agents will, with time, lead to self-propagating, sustainable fire ant control.

Expressed sequence tags (ESTs) have been shown to be an effective method of pathogen gene discovery in insects (Hunter et al., 2006; Hunnicutt et al., 2006). An expression library was created and partially sequenced from a fire ant colony with the intention of virus discovery through homologous gene identification A secondary objective, and added benefit of the study, was the identification of genes from *S. invicta* and other associated pathogens providing a resource for further gene characterization.

2. Methods

2.1. Ants

A single monogyne colony of *S. invicta* was used as the source of mRNA for expression library construction. All developmental stages were included in the mRNA preparation, including eggs, larvae, pupae, workers, male and female alates, sexual larvae and pupae, and the queen. The colony was known to be infected with the microsporidian, *Thelohania solenopsae*.

2.2. mRNA extraction and purification

RNA was extracted according to the method of Salzman et al. (1999). Briefly, the entire ant colony (22.5 g) was homogenized in 150 ml of a homogenization solution (4 M guanidinium isothiocyanate, 0.025 M sodium citrate, 0.5% sodium lauroyl sarcosine, 1.5 ml of 2-mercaptoethanol, and 1.5 g of PVP-40) with a baked pestle and mortar. The homogenate was placed into an RNase-free flask and stirred with a stir bar for 10 min. Acid phenol, pH 4.3, (75 ml) was added to the homogenate and allowed to stir for 10 min. Chloroform: isoamyl alcohol, pH 8.0, (24:1, 75 ml), was added to the homogenate and allowed to stir for another 10 min. Aliquots of the homogenate (~35 ml) were added to eight 40 ml Oakridge tubes and centrifuged for 10 min at 12,000*g*. The aqueous layer was transferred to 40 ml centrifuge tubes. Sodium acetate (2 M) was added at a 10% rate and the sample tubes were inverted several times to ensure distribution of the sodium acetate. One volume of isopropanol (at room temperature) was added and the sample was mixed by inversion. RNA was allowed to precipitate overnight at −20 °C. The samples were centrifuged for 20 min at 13,000*g* (4 °C). The pellet was washed with ice-cold 70% ethanol and centrifuged for 10 min at 12,000*g* (4 °C). The ethanol was discarded and the pellets allowed to dry for 15 min at 37 °C and re-suspended in 1.5 ml of DEPC-treated water. The samples were pooled and one volume of lithium chloride added. After incubation overnight at 4 °C, the sample was centrifuged for 20 min at 12,000*g* (4 °C). The pellet was washed with 70% ethanol, dried at 37 °C and re-suspended in 2 ml of DEPC-treated water. The purified total RNA was treated with DNase according to the manufacturer's directions (Invitrogen, Carlsbad, CA). The DNase-treated total RNA samples were pooled and further purified with the MicroPoly(A) Pure RNA extraction protocol following the manufacturer's directions (Ambion, Austin, TX). Purified mRNA was dried and suspended in DEPC-treated water.

2.3. Library construction

A complementary DNA (cDNA) expression library was constructed using the ZAP-cDNA synthesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. Briefly, first strand cDNA was synthesized from polyadenylated mRNA with 5-methyl dCTP and an oligonucleotide primer with an 18-base poly(dT) region appended with an XhoI restriction site. The RNA/DNA hybrid was digested with RNase H and second strand cDNA synthesis was conducted with DNA polymerase I and unmodified dNTPs. EcoRI adaptors were ligated onto the hemimethylated, double-stranded cDNA which was subsequently digested with XhoI producing unidirectional cDNA comprised of an EcoRI restriction site at the 5′ terminus and an XhoI restriction site at the 3′ terminus (relative to the transcript). cDNA was ligated into the Uni-ZAP XR vector and packaged into phage with the Gigapack III Gold packaging kit (Stratagene). Mass excision of the amplified library was carried out using the Ex-Assist (Stratagene) helper phage. Bacterial clones containing the excised pBluescript SK(+) phagemid were recovered by random selection. The bacterial stocks are archived at the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, and the Horticulture Research Laboratory, Fort Pierce, FL.