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New approach for the study of mite reproduction: The first transcriptome analysis of a mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae)

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ABSTRACT

Many species of mites and ticks are of agricultural and medical importance. Much can be learned from the study of transcriptomes of acarines which can generate DNA-sequence information of potential target genes for the control of acarine pests. High throughput transcriptome sequencing can also yield sequences of genes critical during physiological processes poorly understood in acarines, i.e., the regulation of female reproduction in mites. The predatory mite, *Phytoseiulus persimilis*, was selected to conduct a transcriptome analysis using 454 pyrosequencing. The objective of this project was to obtain DNA-sequence information of expressed genes from *P. persimilis* with special interest in sequences corresponding to vitellogenin (Vg) and the vitellogenin receptor (VgR). These genes are critical to the understanding of vitellogenesis, and they will facilitate the study of the regulation of mite female reproduction. A total of 12,556 contiguous sequences (contigs) were assembled with an average size of 935 bp. From these sequences, the putative translated peptides of 11 contigs were similar in amino acid sequences to other arthropod Vgs, while 6 were similar to VgRs. We selected some of these sequences to conduct stage-specific expression studies to further determine their function.

1. Introduction

The Acari are an important and diverse group of arthropods that include pests of importance to human health, livestock and agricultural crops, such as ticks, chiggers, spider mites, and other parasitic and/or predatory species (Klompen et al., 1996; Huffaker et al., 1969). The biology, ecology and behavior of several species of ticks and mites have been extensively studied, but studies at the genomic and transcriptomic levels are scarce. Recently, the genome of the deer tick, *Ixodes scapularis*, was sequenced and is now available at the VectorBase website (www.vectorbase.org). Although much can be learned about the acarines by the study of the *I. scapularis* genome, ticks only represent one group of the Acari (suborder Ixodida), and there are great differences even among ticks as well as expected differences between ticks and mites. For example, the size of the deer tick genome is close to 1.7 Gbp (www.vectorbase.org) while mite genomes range between 75 and 90 Mbp (Hoy, 2009; Grbic et al., 2007). There are plans to sequence the genome of the predatory mite, *Metaseiulus occidentalis* (Hoy, 2009), in the near future, and there is a genome sequence project for the twospotted spider mite *Tetranychus urticae* (Grbic et al., 2007), but these resources are not currently available.

The predatory mite, *P. persimilis*, belongs to the order Parasitiformes that also contains ticks (Krantz, 1978). This mite is readily available and commercialized as a biological control agent for spider mites (Cote et al., 2002). Phytoseiid mites are mostly found in the foliage of herbaceous plants and trees where they feed on all life stages of their prey (McMurtry and Croft, 1997). The regulation of female reproduction in this species or mites in general has not been studied in depth (Cabrera et al., 2009). The original idea that mites regulate their reproduction similarly to insects with increasing levels of juvenile hormone (JH) may not be accurate. Cabrera et al. (2009) recently reviewed the literature and proposed a new model where ecdysteroids and not JH regulate

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vitellogenesis in mites. Obtaining DNA-sequence information of vitellogenin (Vg) and the vitellogenin receptor (VgR) will help to advance the study of the regulation of female reproduction in mites.

We selected *P. persimilis* for 454 transcriptome pyrosequencing. To our knowledge, this is the first effort to sequence a transcriptome of a mite with a high-throughput technology like 454. A recent search in GenBank (January 2010, www. ncbi.nlm.nih.gov) showed that while there are currently 97 gene and 26 protein sequences of *P. persimilis*, none of them correspond to Vg or VgR. The objective of this study was to generate DNA-sequence information of expressed genes from the predatory mite *P. persimilis*, obtain partial or complete sequences of the Vg and VgR genes, and to begin learning more about message function from the sequences obtained. The Vg and VgR genes can be used in the future to study the regulation of female reproduction in mites.

2. Materials and methods

2.1. Mites

Predatory mites, *P. persimilis* (Acari: Phytoseiidae), were purchased from IPM Laboratories, Inc. (Locke, NY). Mobile stages including the larva, protonymph, deutonymph, and adult (male and female) were collected in 1.5-ml Eppendorf tubes and weighed. The majority of collected mites were immature stages, followed by female adults and very few males. Each sample consisted of 1–1.5 mg of all mobile mite stages. Immediately afterward, 50 μl of TRI Reagent (Sigma–Aldrich, St. Louis, MO) was added to each sample and the mites homogenized using a Fisherbrand disposable pestle system (Fisher Scientific, Pittsburgh, PA). The samples were immediately stored at −80 °C until the construction of the cDNA library.

2.2. Construction of the mite cDNA library for 454 pyrosequencing

Six samples of mites previously homogenized in TRI Reagent were combined to prepare a cDNA library; all mites combined weighed 7 mg. The procedure for the construction of the mite cDNA library was described before by Donohue et al. (2010). Following the TRI Reagent protocol for total RNA extraction, we obtained approximately 80.6 μg of total RNA collected in 50 μl of 100 μM aurintricarboxylic acid (ATA) known to prevent RNA degradation (Hallick et al., 1977). An Oligotex mRNA isolation kit (Qiagen, Valencia, CA) was used to isolate mRNA according to the manufacturer's recommendations. The mRNA was concentrated by precipitation with 3 M potassium acetate and ethanol and re-suspended in 2 μl of RNAse free water.

First strand cDNA synthesis was performed by combining the mRNA with 10 pmol of a modified 3′ reverse transcription primer (5′-ATTCTAGAGACCGAGGCGGCCGACATGT(4)GT(9)CT(10)VN-3′) (Beldade et al., 2006) and 10 pmol of SMART IV oligo (5′-AAGCAGTGGTATCAACGCAGAGTGGCCATTACGGCCGGG-3′) (Zhu et al., 2001) followed by incubation at 72 °C for 2 min. Afterwards, the tube containing mRNA and primers was placed immediately on ice and the following reagents were added: 2 μl of 5× first-strand buffer, 1 μl of 20 mM dithiothreitol (DTT), 1 μl dNTP mix (10 mM each), 1 μl RNAse Out (40 U/μl) and 1 μl of Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) followed by incubation at 42 °C for 90 min. The reaction was diluted to 30 μl with 1× TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5), incubated at 70 °C for 15 min to stop the reaction, and then stored at −80 °C until used.

Prior to second strand cDNA synthesis, the number of PCR cycles was optimized. A sample of 5 μl of first strand cDNA was used as DNA-template and combined with 10 pmol of modified 3′ PCR primer (5′-ATTCTAGAGGCCGAGGCGGCCGACATGT(4)GTCT-(4)GTTCTGT(3)CT(4)VN-3′) (Beldade et al., 2006), 10 pmol of 5′ PCR primer (5′-AAGCAGTGGTATCAACGCAGAGT-3′) (Zhu et al., 2001), 5 μl 10× reaction buffer, 2 μl $MgSO_4$, 1 μl dNTP mix (10 mM each), 0.4 μl Platinum HiFi Taq Polymerase (Invitrogen) and 34.6 μl water. The thermal cycler (PTC-100™ Peltier Thermal Cycler, MJ Research, Waltham, MA) was programmed with the following conditions: 94 °C for 2 min, followed by 25 cycles with 94 °C for 20 s, 65 °C for 20 s and 68 °C for 6 min. Aliquots of 5 μl from cycles 12, 15, 18, 22 and 25 were taken and evaluated with a 1% agarose gel (0.5 μg EtBr/ml agarose solution, 85 V for 1 h). We selected 25 cycles and ran 5 additional reactions to generate enough cDNA for sequencing with the same thermal cycler program previously described. The cDNA was purified from each reaction using a PCR purification kit (Qiagen) according to the manufacturer's recommendations. The samples were combined and the concentration measured using a nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA). Five μg of purified cDNA was submitted to the Genomic Sciences Laboratory at North Carolina State University for pyrosequencing with the 454 technology. The cDNA library was further prepared for pyrosequencing on the GS-FLX sequencer with titanium reagents (Roche, Indianapolis, IN) according to the manufacturer's recommendations as described by Margulies et al. (2005).

2.3. Bioinformatics

The processing of the individual reads from the 454 sequencing was conducted as described by Donohue et al. (2010). The GS Assembler ver. 1.1.02.15 (Roche) removed the primer sequence contamination and assembled sequencing reads with the following default parameters: seed step: 12, seed length: 16, seed count: 1, minimum overlap length: 40, minimum overlap identity: 90%, alignment identity score: 2, and alignment difference score: −3. Assembled contiguous sequences, referred to as contigs, were identified using the Tera-BLASTX algorithm with DeCypher (TimeLogic, Carlsbad, CA) against the non-redundant (nr) and expressed sequence tag (est) databases in GenBank (downloaded February 2009). Contigs were also compared to the *Ixodes scapularis* genome available at the VectorBase website (www.vectorbase.org). The gene ontology (GO) analysis was conducted using the program Blast2GO (www.blast2go.org, Valencia, Spain) on November 2009. Individual contigs with at least one match similar to vitellogenin (Vg) and the vitellogenin receptor (VgR) were further studied using the Open Reading Frame tool in GenBank to retrieve more information about the putative translated peptides. Alignments were conducted with ClustalW (www.ebi.ac.uk/clustalw). MEGA4 was used to generate an evolutionary tree with the *P. persimilis* Vg contigs 00558, 08149, 11791 and 12365 (contigs 10299 and 11824 not included due to their short length), tick carrier proteins, and tick and insect Vgs. The tick messages were obtained from the non-redundant database in GenBank. We included 5 sequences of insect melanization-related proteins and hypothetical proteins (unknown) as the out-group. The analysis conducted was Neighbor-Joining with the Poisson correction model and 1000 replicates for the bootstrap test.

2.4. Stage-specific expression studies

Six contigs with similarity to arthropod Vgs and three to arthropod VgRs were selected for stage-specific expression studies. These contigs were selected based on one or more of the following criteria: high number of individual reads from which the contig was constructed, the presence of conserved motifs and length. The selected putative Vg contigs were 00558, 08149, 10299, 11791, 11824 and 12365, and the selected putative