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ESTIMATING BACTERIAL DIVERSITY IN *SCIRTOTHRIPS DORSALIS* (THYSANOPTERA: THRIPIDAE) VIA NEXT GENERATION SEQUENCING

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ABSTRACT

The last 2 decades have produced a better understanding of insect-microbial associations and yielded some important opportunities for insect control. However, most of our knowledge comes from model systems. Thrips (Thysanoptera: Thripidae) have been understudied despite their global importance as invasive species, plant pests and disease vectors. Using a culture and primer independent next-generation sequencing and metagenomics pipeline, we surveyed the bacteria of the globally important pest, *Scirtothrips dorsalis* Hood. The most abundant bacterial phyla identified were Actinobacteria and Proteobacteria and the most abundant genera were *Propionibacterium, Stenotrophomonas, and Pseudomonas*. A total of 189 genera of bacteria were identified. The absence of any vertically transferred symbiont taxa commonly found in insects is consistent with other studies suggesting that thrips primarilly acquire resident microbes from their environment. This does not preclude a possible beneficial/intimate association between *S. dorsalis* and the dominant taxa identified and future work should determine the nature of these associations.

Key Words: Next Generation Sequencing, Metagenomics, chilli thrips

RESUMEN

Durante las últimas dos decadas se ha alcanzado una mejor comprensión acerca de la asociación insecto-microbio, lo cual ha entregado importantes oportunidades para el control de insectos. Sin embargo, la mayor parte de nuestro conocimiento proviene de sistemas modelo, en que los Trips (Thysanoptera: Thripidae) no han sido estudiados en profundidad, a pesar de su importancia como especie invasiva, plaga de plantas y vector de enfermedades. Utilizando métodos de sequenciación de última generación sin necesidad de primers o cultivos, asi como metagenómica, hemos sondeado la bacteria *Scirtothrips dorsalis* Hood, una plaga de importancia mundial. Las phyla bacteriales mas abundantes identificadas fueron Actinobacteria and Proteobacteria, mientras que los géneros fueron *Propionibacterium*, *Stenotrophomonas* y *Pseudomonas*. Un total de 189 géneros de bacteria fueron identificados. La ausencia de cualquier tipo de taxa simbionte transferida verticalmente, como aquella encontrada frecuentemente en insectos, es consistente con otros estudios que sugieren que los microbios residentes en trips provienen principalmente del medio ambiente. Ésto no excluye una posible asociación íntima/beneficiaria entre *S. dorsalis* y la taxa domimante identificada. La naturaleza de estas asociaciones deberá ser determinada en futuros estudios.

Palabras Clave: Sequenciacion de última generación, Metagenómica, *Scirtothrips dorsalis*

Thrips, order Thysanoptera, are emerging as a globally important group of plant pests, damaging crops through direct feeding and transmission of tospoviruses and non-viral diseases (Morse & Hoddle 2006). Only a few hundred of the 5,500 identified thrips species are pests (Brunner et al. 2002), and of these, only 14 are documented virus vectors (Riley et al. 2011). Chilli thrips *Scirtothrips dorsalis* is both a virus vector (Chu et al. 2001; Meena et al. 2005; Gopal et al. 2010) and a pest of many crops around the world including tea (Saha & Mukhopadhyay 2013), mango (Aliakbarpour & Md. Rawi 2012; Choi et al. 2013), roses (Hegde et al. 2011; Mannion et al. 2013), and citrus (Gao et al. 2012; Hyun et al. 2012). It is a highly polyphagous species, feeding on >100 plant species in 40 different families, many containing important U.S. crops (Hodges et al. 2007; Kumar et al. 2013). *S. dorsalis* is globally invasive and has been established in Florida and Texas since 2005 (Mannion et al. 2013).

Bacterial associations with insects are ubiquitous and are often beneficial to the insect (Duron & Hurst 2013). Bacteria have been implicated in the manipulation of reproduction (Duron et al. 2008), body color (Tsuchida et al. 2010), disease transmission (Weiss & Aksoy 2009), development (Chouaia et al. 2012), and protection from parasites (Brownlie & Johnson 2009). Thrips on the whole have received little attention regarding this important aspect of arthropod ecology. Previous work has largely focused on the most frequently encountered bacterium (identified as a near-*Erwinia* species) within a single thrips species, the Western flower thrips *Frankliniella occidentalis* (de Vries et al. 2001a; de Vries et al. 2001b; de Vries et al. 2004; de Vries et al. 2006; Chanbusarakum & Ullman 2008, 2009; de Vries et al. 2012) with a few studies on other species (Wells et al. 2002; Gitaitis et al. 2003; de Vries et al. 2008).

In this study, next generation semiconductor sequencing was conducted on invasive chilli thrips in Florida from which we present the first metagenomic survey of any thrips. This expands our understanding of the bacterial symbioses of thrips in general and is a first step toward a bacterial transfection biocontrol strategy for this important pest.

Methods

Next Generation Sequencing

A single DNA extraction from 97 adult and nymph *S. dorsalis* was made using a DNeasy™ Blood and Tissue kit (Qiagen™ , Valencia, California). Eluted DNA was concentrated to a volume of ~10 mL in a SpeedVac™ DNA 110 Concentrator (Savant, Farmingdale, New York), subjected to electrophoresis on a 1.5% agarose gel, and high molecular weight genomic DNA (> 10Kb) was cut out and purified with a Nucleospin Clean-up kit (Macherey-Nagel, Pennsylvania, USA). One hundred ng of purified DNA was used for library preparation using an Ion Xpress™ Plus Fragment Library Kit. End repair, adapter ligation, size selection, nick repair and amplification (16 cycles) were performed as described in the Ion Torrent protocol associated with the kit. 300 bp fragments were isolated using a SizeSelect 2% Gel in an E-Gel electrophoresis system (all Life Technologies, California, USA).

The Agilent 2100 Bioanalyzer and the associated High Sensitivity DNA kit (Agilent Technologies, Englewood, Colorado) were used to determine quality and concentration of the library. The amount of library required for template preparation was calculated using the Template Dilution Factor calculation described in the protocol. Next-generation sequencing was conducted using the Ion Torrent Personal Genome Machine using an Ion PGM 200 Sequencing kit following sequencing template preparation with the OneTouch™ 2 System and Ion PGM™ Template OT2 200 Kit according to manufacturer's instructions (all products: Life Technologies). The library was sequenced on a single Ion 318 semiconductor chip (Life Technologies) with a barcode.

Contig Assembly and Metagenomics

Contigs were assembled from the raw reads using the following parameters in Geneious v6; word length, 12; index word length, 11; maximum gap size, 5; maximum gaps per read, 10%; maximum mismatches, 5%; and maximum ambiguity, 16. Assembled contigs were compared against the NCBI nt database (Retrieved 9-XII-2012) and to a custom combined LSU and SSU Silva database (Release SSURef_111 and LSURef_111) (Quast et al. 2013) with BLASTN 2.2.27+ (Camacho et al. 2009) with an *E*-value of 1e-10 with BLAST data outputted in XML format. XML outputs from BLAST coupled with the corresponding assembly input data were provided as input to MEGAN v4.70.4 (Huson et al. 2011) and analyzed with default lowest common ancestor parameters. The minimum support filter was set to one ensuring rare taxa were represented. Contigs compared to the Silva database were mapped to taxa using the silva_2_ncbi roadmap. The taxonomic tree generated by MEGAN was manually parsed to present the data at the level of microbial *Genus.* To accomplish this, the tree was uncollapsed at the level of *Genus,* followed by the collapse of all non-microbial taxa at the level of Kingdom. Using the summarize feature of MEGAN the number of both direct and summed (assigned to a more specific taxon) contigs were exported in a tabular format, including non-microbial Kingdoms, microbial *Genera,* as well as nodes representing low complexity sequences, sequences which had no BLAST hit, and those not assigned to a given taxa. Contigs assigned to nodes below the *Genus* threshold and lost/not processed contigs were calculated using total reads in raw files, the number of reads placed in the MEGAN root taxon, and the total assigned to selected nodes. Summary data was exported to Microsoft Excel and graphed. A listing of the various implicated bacterial species is provided in supplementary material for this article in Suppl. Table 1 in Florida Entomologist 97(2) (2014) online at http://purl.fcla.edu/fcla/entomologist/browse .

Results and Discussion

The sequencing yield was 6,204,869 million raw reads averaging 164 bases. From these reads, 615,073 contigs were assembled averaging 483 bases. More than 99.99% of contigs were processed successfully through the metagenomics pipeline. 28,416 contigs were identified as having microbial origin and 189 bacterial genera were identified as the best match for a minimum of 4 different contigs (Table 1, Suppl. Table 1). The relative abundance of sequences from identified genera was highest for the phyla Actinobacteria, and Proteobacteria (Fig. 1). This is consistent with the dominant bacterial phyla found for other animals (Jones et al. 2013).

Table 1. Abundance of the 15 most commonly identified bacterial genera in *Scirtothrips dorsalis*. The remaining 174 species are listed in suppl. Table 1.

Genus(# Matches)	Phylum
Propionibacterium(5072)[1]	Actinobacteria
Stenotrophomonas(3143)[1+]	Proteobacteria
Pseudomonas(2717)[1+]	Proteobacteria
Methylobacterium(827)[1+]	Proteobacteria
Ralstonia(614)[1]	Proteobacteria
Streptococcus(455)[1+]	Firmicutes
Deinococcus(398)[1+]	Deinococcus-Thermus
Bradyrhizobium(350)[1+]	Proteobacteria
Enterobacter(338)[1*+]	Proteobacteria
Achromobacter(289)[1+]	Proteobacteria
Mycobacterium(286)[2]	Actinobacteria
Nocardioides(275)[2]	Actinobacteria
Rothia(270)	Actinobacteria
Sphingomonas(270)[1+]	Proteobacteria
Sphingobium(268)[3]	Proteobacteria

[1-3]Genus previously documented associated with insects:
[1](Minard et al. 2013)
[2](Hail et al. 2012)
[3](Shelomi et al. 2013)
[*]Order: Enterobacteriales
[+]Also hit in Silva database

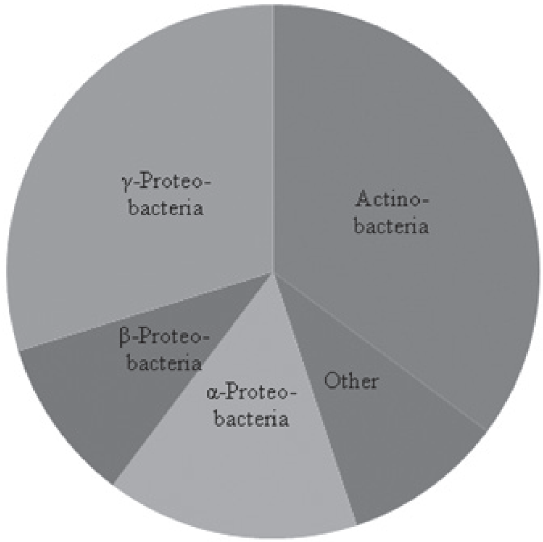

Fig. 1. Relative abundance of dominant bacterial taxa in *Scirtothrips dorsalis*. Abundance based on 23,068 contigs classified to 189 genera (supplimental table). Genera were included if identified as the best match for >3 contigs.

Eight genera identified by this study have previously been associated with other thrips species. *Erwinia*, *Pantoea*, *Enterobacter*, *Serratia*, *Escherichia* and *Pseudomonas* have been identified internally from thrips (de Vries et al. 2001a; Gitaitis et al. 2003; Chanbusarakum & Ullman 2008; de Vries et al. 2008; de Vries et al. 2012). *Erwinia herbicola* and *Enterobacter agglomerans* have been synonymized with *Pantoea agglomerans* so these 3 genera identified in chilli thrips could represent a single taxon found in association with several insects (Medina et al. 2011). *Agrobacterium*, *Methylobacterium*, *Pseudomonas* and *Escherichia* have been isolated from the exoskeleton of *Sericothrips staphylinus* (Yamoah et al. 2008).

Many of the genera identified by this study have members with documented insect associations (Table 1, Suppl. Table 1), though a thorough literature search on all 189 genera was not conducted. Notably absent from the list are heritable genera reported as globally common in insects by Duron & Hurst (2013), *Arsenophonus*, *Wolbachia*, *Rickettsia*, *Spiroplasma*, and *Cardinium*. In the 3 thrips species with literature records of endosymbiotic bacterial associations, it has generally been argued that the bacteria are facultative, and acquired from the environment through feeding (de Vries et al. 2001b; Gitaitis et al. 2003; de Vries et al. 2008). Our results suggest this may also be the case for *S. dorsalis*. In *Frankliniella occidentalis*, a geographically ubiquitous association with a near *Erwinia* species provisionally identified as

Pantoea agglomerans, is promoted behaviorally since thrips individuals prefer thrips-damaged leaves (de Vries et al. 2006). Future work should investigate whether any of the dominant bacteria identified in *S. dorsalis* could be maintained in a similar fashion as this could provide a mechanism for the spread of transfected bacteria through the thrips population for the purpose of biological control.

There are several factors limiting the inferences that can be drawn from this dataset. First, we are unable to distinguish internal symbionts from those associated with the surface of the insect or possible contaminants (see Hail et al. 2012). Second, we cannot gauge the bacterial communities of individuals or life-stages. Both of these limitations can be overcome within the context of this sequencing and bioinformatics framework at an increased cost. That being said, the abundance of genera identified in the order: Enterobacteriales and those with documented insect associations (Table 1, Suppl. Table 1) argue that many of the identified taxa are thrips associated.

Massively parallel, high throughput, primer independent sequencing has some benefits over bacterial culturing, traditional PCR with cloning, and target enriched next-generation sequencing for estimating bacterial diversity. It not only does away with the assumption of culturability, but also with the assumption of equivalent primer-template compatibility for all bacteria. As such, it has the ability to recover taxa that may not have been detected with primer dependent methods (Mao et al. 2012). The primer independent method presented here identified a large number of bacterial taxa, free of these assumptions, for an insect with no prior information about its microbiome.

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