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Special Collection

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Differences in Microbiota Between Two Multilocus Lineages of the Sugarcane Aphid (*Melanaphis sacchari*) in the Continental United States

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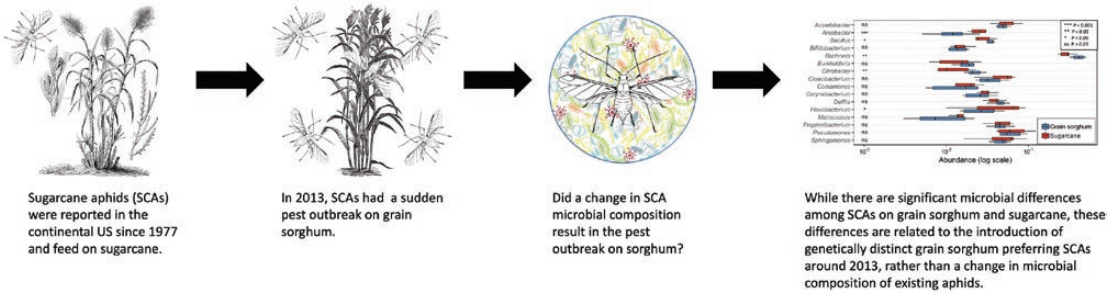
Abstract

The sugarcane aphid (SCA), *Melanaphis Sacchari* (Zehntner) (Hemiptera: Aphididae), has been considered an invasive pest of sugarcane in the continental United States since 1977. Then, in 2013, SCA abruptly became a serious pest of U.S. sorghum and is now a sorghum pest in 22 states across the continental United States. Changes in insect-associated microbial community composition are known to influence host-plant range in aphids. In this study, we assessed whether changes in microbiota composition may explain the SCA outbreak in U.S. sorghum. We characterized the SCA bacterial microbiota on sugarcane and grain sorghum in four U.S. states, using a metabarcoding approach. In addition, we used taxon-specific polymerase chain reaction (PCR) primers to screen for bacteria commonly reported in aphid species. As anticipated, all SCA harbored the primary aphid endosymbiont *Buchnera aphidicola,* an obligate mutualistic bacterial symbiont. Interestingly, none of the secondary symbionts, facultative bacteria typically associated with aphids (e.g., *Arsenophonus*, *Hamiltonella*, *Regiella*) were present in either the metabarcoding data or PCR screens (with the exception of *Rickettsiella* and *Serratia,* which were detected by metabarcoding at low abundances <1%). However, our metabarcoding detected bacteria not previously identified in aphids (*Arcobacter*, *Bifidobacterium*, *Citrobacter*). Lastly, we found microbial host-associated differentiation in aphids that seems to correspond to genetically distinct aphid lineages that prefer to feed on grain sorghum (MLL-F) versus sugarcane (MLL-D).

Graphical abstract



Key words: bacterial host-associated differentiation, bacterial symbiont, invasive insect, metabarcoding

The sugarcane aphid (SCA), *Melanaphis Sacchari* (Zehntner) (Hemiptera: Aphididae), is a globally distributed species that feeds on members of Poaceae including sugarcane, sorghum, rice, millet, corn, and wild grasses (Singh et al. 2004). In most parts of the world (e.g., Asia, Australia, the Americas, and Africa), SCA is considered a pest of sorghum (Singh et al. 2004). Its center of origin is currently unknown, but it is hypothesized to be either in central or northern Africa or in Asia (Nibouche et al. 2014). In regions of the world where both sugarcane (*Saccharum officinarum* and *Saccharum* spp. Linnaeus [Poales: Poaceae]) and grain sorghum (*Sorghum bicolor*, L. Moench [Poales: Poaceae]) are grown, SCA is reported to have higher abundances on grain sorghum than on sugarcane and exhibits preference for sorghum over sugarcane (Nibouche et al. 2015). SCA was first reported on sugarcane in the continental United States in Florida during 1977 (Mead 1978) and in Louisiana during 1999 (White et al. 2001). Despite the presence of SCA on commercial sugarcane, SCA was not a sorghum pest in the continental United States (Mead 1978, Hall 1987, Denmark 1988, Armstrong et al. 2015, Medina et al. 2017) until spring 2013 when SCA was reported on grain sorghum (Bowling et al. 2016). SCA damage to sorghum is caused by feeding activity and honeydew production, which combined can decrease crop yields and harvesting efficiency (Bowling et al. 2016, Zapata et al. 2016). Damage estimates in U.S. grain sorghum range from 20 to 100% crop loss (Villanueva et al. 2014, Kerns 2015, Zapata et al. 2016) plus the additional financial burden incurred by pest management efforts (Zapata et al. 2016).

Considering the fact that SCA was already present in U.S. sugarcane, a host-switch could have occurred due to a change in SCA's symbiotic bacteria. Symbiotic bacteria are a part of an insect's microbiota defined as a collection of microorganisms (e.g., bacteria, fungi, protists, and viruses) contained within and on the surface of an insect host. Similar to the important role microbiomes play in humans (Hartstra et al. 2015, Findley et al. 2016, Marchesi et al. 2016), the bacterial composition in aphids can influence their health, resource use, and vector potential (Oliver et al. 2003, Oliver et al. 2010, Lukasik et al. 2013). Symbiotic bacteria may influence insect host-range through nutritional supplementation (Hosokawa et al. 2007) or by helping their insect hosts withstand plant defenses (Adams et al. 2013, Ceja-Navarro et al. 2015, Hammer and Bowers 2015). For example, when the kudzu bug (*Megacopta cribraria*) invaded the United States, it was able to switch from kudzu to soybean because of its association with a bacterial strain of the obligate symbiont (one required for the insect's survival) Candidatus *Ishikawaella capsulata* (Hosokawa et al. 2007, Brown et al. 2014). Similarly, facultative bacteria (potentially beneficial to the insect, but not essential for survival) in the genus *Arsenophonus* improve cowpea aphids' (*Aphis craccivora*) fitness on locust plants (Wagner et al. 2015), whereas *Regiella insecticola* increases pea aphid fecundity on clover (Leonardo and Muiru 2003, Tsuchida et al. 2004).

Most research on aphid bacterial composition has been conducted on a few well studied species, such as the pea aphid and cowpea aphid through the use of polymerase chain reaction (PCR) with taxon-specific primers (Chen et al. 1996, Darby et al. 2001, Simon et al. 2003, Brady and White 2013, Brady et al. 2014). However, it is important to understand the bacterial composition of nonmodel organisms as they may differ in symbiont composition and those symbionts' biological functions. Although the use of PCR to detect specific taxa remains an effective method for detection of well-known symbionts (e.g., *Hamiltonella defensa*, *Serratia symbiotica*, and *Regiella insecticola*), it requires prior knowledge of the bacterial taxa and taxon-specific DNA sequences to be used for detection (Munson et al. 1991, Sandstrom et al. 2001, Russell et al. 2003, Oliver et al. 2006). Another approach called barcoding uses general or universal PCR primers to amplify common genomic regions from a variety of organisms. After using barcoding to sequence single fragments of DNA or RNA, the nucleotide composition can be used as a proxy for organism identification. The advent of high-throughput sequencing (HTS) technologies, with the initial large data generating technologies referred to as next-generation sequencing (NGS), allow for the massive parallel sequencing of short DNA fragments pushing the boundaries of DNA barcoding and allowing the reconstruction of entire communities of organisms (Abdelfattah et al. 2018). With metabarcoding, a combination of PCR identification and high throughput sequencing, most of the bacteria harbored by an insect can be identified without any prior knowledge of what an insect may harbor and without the need to cultivate thousands of bacterial colonies or to clone thousands of DNA fragments (Mardis 2008, Malacrinò 2018).

Some of the earliest studies of aphids using 454 pyrosequencing were done on the microbial symbionts in cowpea aphids (Brady and White 2013), soybean aphid (Bansal et al. 2014), and pea aphids (Russell et al. 2013, Gauthier et al. 2015). Recently, metabarcoding has been used to identify bacteria that were not previously associated with aphids. Metabarcoding has allowed for the identification of potential symbionts that might otherwise go unnoticed with the screening of only specific symbionts (Bansal et al. 2014, Gauthier et al. 2015, Jousselin et al. 2016, Fakhour et al. 2018).

The objective of our study was to characterize the SCA bacterial microbiota from aphids collected from sorghum and sugarcane using PCR and metabarcoding. In addition, we sought to determine whether a change in SCA microbiota supports a host–plant shift, from sugarcane to grain sorghum.

Materials and Methods

Field Collections

Specimens of sugarcane aphid were collected from grain sorghum and sugarcane in four different states (i.e., Florida, Alabama, Louisiana, and Texas) in the United States between 2014 and 2015 and from sorghum in South Africa in 2014 (Table 1). Aphids within

Table 1. Number of sugarcane aphids that were pooled for each host plant and state combination used in microbial analyses

Host Plant	Location and year	Number of SCA pooled
Grain Sorghum		
	Alabama 2014	18
	Lab Colony 2015–2017	25
	Lab Colony 2015 (nonsurface sterilized)	30
	Florida 2014	24
	Florida 2015	30
	Louisiana post 2013	17
	South Africa 2013	19
	Texas 2013	21
Sugarcane		
	Alabama 2014	8
	Florida 2013 (nonsurface sterilized)	9
	Florida 2014	21
	Louisiana 2007–2009	7
	Louisiana 2015	20
	Texas 2015	20

Within each county, each aphid was collected at least 1 km away from each other (except for lab colony aphids). In total, 14 pooled samples were used for analysis.

each state were collected on both grain sorghum and sugarcane from as many counties as possible, collecting specimens from fields at least 1 km apart from each other to minimize the chance of sampling siblings. We also added SCA samples from Louisiana sugarcane collected between 2007 and 2009, before the 2013 SCA invasion on sorghum in United States (Table 1). Aphids were killed in 95% ethanol and stored at 4°C.

DNA Extraction

Nymphs and apterous adults from each combination of host plant species and location (Table 1) were pooled together and surface sterilized before DNA extraction (Meyer and Hoy 2008, Medina et al. 2011), yielding a total of 14 samples (each containing an average of approximately 20 individuals). DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA) following the standard protocol recommended by the manufacturer. DNA concentration and quality were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). Aliquots from those same samples were also used for both taxon-specific PCR and metabarcoding.

PCR for Specific Aphid Symbionts

We used taxon-specific PCR primers to screen for nine bacterial genera found in other aphid species. These bacterial genera included: *Arsenophonus, Cardinium, Hamiltonella, Regiella, Rickettsia, Rickettsiella, Serratia, Spiroplasma,* and *Wolbachia* (Fukatsu et al. 2001, Russell and Moran 2006, Oliver et al. 2010, Brady and White 2013). PCR reactions were run on a MyCycler Thermal Cycler (Bio-Rad, Hercules, CA) in a total volume of 10 µl containing: 2.5 mM MgCl2, 10X Taq reaction buffer (NEB), 2.5 mM dNTPs (Omega Bio-Tek, Norcross, GA, USA), 5 µM of forward and reverse primers, 0.1 µl of 5U/µl *Taq* DNA Polymerase (NEB, Ipswich, MA), and 2 µl of DNA template. The list of taxon-specific primers and annealing temperatures can be found in Supp Table 1 (online only). All diagnostics included positive (i.e., symbiont-positive specimens known to host the bacteria of interest) and negative controls (i.e., nuclease-free water). PCR products were visualized on a 1% agarose gel stained with GelRed (Biotium, Fremont, CA) under UV light. For samples that produced products of the expected size, we re-ran PCRs with a total volume of 25 µl and either purified the product using the GenCatch PCR Cleanup Kit (Epoch Life Sciences, Missouri City, TX) or in the case of double bands appearing on a gel, the band of interest (i.e., a band matching the correct size compared to the ladder) was excised and purified using the GenCatch Gel Extraction Kit (Epoch Life Sciences, Missouri City, TX). Purified products were sent to an offsite facility for Sanger sequencing (GENEWIZ, South Plainfield, NJ). Resulting sequences were searched by MegaBLAST in the GenBank database default parameters, and only sequences returning ≥97% similarity to the expected bacterial genus were considered for inclusion in bacterial presence analyses.

16S rRNA Metabarcoding

DNA samples were sent to the Molecular Research DNA Lab (MR. DNA, Shallowater, TX) for metabarcoding analyses targeting the bacterial V3-V4 16S rRNA bacterial region (Herlemann et al. 2011, Su et al. 2016). Samples were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA) using the MiSeq Reagent Kit v3 300PE chemistry. Together with experimental samples, we submitted two negative controls to identify potential environmental bacterial contaminants (Salter et al. 2014). One of the negative controls consisted of a sterilized water sample run with all the chemicals and the same protocol used for DNA extraction without any aphid DNA (Salter et al. 2014, Malacrinò et al. 2018). The second negative control consisted of a single pool of all the last wash liquids obtained from the surface sterilization of insects, which was used for the identification of bacteria likely to be potential surface contaminants.

Data Analysis

Data handling was carried out using QIIME 1.9 (Caporaso et al. 2010, Caporaso et al. 2012), quality-filtering reads (Phred ≥ 25), binning OTUs using open-reference OTU-picking through UCLUST algorithm, and discarding chimeric sequences discovered with USEARCH 6.1 (Edgar 2010). OTUs were taxonomically assigned through the BLAST method using Greengenes database for 16S rRNA (Caporaso et al. 2012). The OTU table was then filtered to remove all singletons, OTUs coming from amplification of chloroplast DNA, and those clearly belonging to contaminants (i.e., *Gardnerella*, *Granulicatella*, *Haemophilus*, *Leptotrichia*, *Prevotella*, *Ruminococcus*, *Staphylococcus*, and *Streptococcus*). The two negative controls used in this study clustered apart from the aphid samples and allowed us to further clean our dataset from contaminants. Using a statistical approach to discover potential contaminants (Davis et al. 2018; Raw reads are available at NCBI SRA under accession number PRJNA419038), we further removed 30 OTUs from our samples. In addition, we subtracted from each sample the quantity of OTU reads found in both control types we used, under the assumption that they could be surface contaminants. Since each sample had a different sampling depth and library size, before subtraction we normalized counts using the Variance Stabilizing Transformation algorithm from DESeq2 package (Love et al. 2014) and removed any batch effects using package limma (Ritchie et al. 2015). Negative values were then converted to zero, and we performed another round of singleton removal.

Differences in the structure of microbial communities between aphids collected from different host plants was assessed through a PERMANOVA analysis (999 permutations) using host plant as a fixed variable and sampling location to stratify permutations as a random variable to account for geographical variability. Distances between samples were calculated through a weighted UniFrac matrix and then visualized using Non-Metric Multidimensional Scaling (NMDS). Differences in relative abundance of each bacterial genus between samples from different host plants were tested through a mixed-effect model (*host plant* as fixed effect, and *location* as random variable) with *lmer* function (lme4 R package), using False Discovery Rate (FDR) correction for multiple comparisons. All analyses were performed using R statistical software (R Core Team 2013) with the packages vegan (Dixon 2003), phyloseq (McMurdie and Holmes 2013), and picante (Kembel et al. 2010).

Results

Bacterial Identification with PCR

PCR analyses did not detect bacterial symbionts commonly reported in other aphid species (i.e., *Arsenophonus*, *Hamiltonella*, *Regiella*, *Rickettsia*, *Rickettsiella*, *Spiroplasma,* and *Wolbachia*) see the example for *H. defensa* (Supp Fig. S2 (online only)). These bacteria were also not identified using metabarcoding, with the exception of *Rickettsiella* and *Serratia*, which were also detected in low abundances using metabarcoding.

Microbiota and Host-Association with Metabarcoding

In total, 1,081,493 reads (with a sample average of 86,565 ± 18,914 SE paired end reads) were obtained from Illumina MiSeq, which clustered into 267 OTUs. Through a multivariate approach,

we found host-associated differentiation of bacterial communities between aphids collected on sugarcane and grain sorghum ($F_{1,12}$ = 4.73; P = 0.009). The differentiation of the microbial community harbored by SCA clustered by host plant, which is visible in the NMDS plot (Fig. 1).

As anticipated, the majority of the bacterial reads belonged to the obligate aphid bacterial symbiont *Buchnera*. The raw unweighted percentages for *Buchnera* averaged between 90 and 99%, which overpowers the signal of less prominent bacteria if uncorrected. Consequently, all values are reported in weighted relative abundances. *Buchnera* composed a greater proportion of the bacterial community in aphids feeding on sorghum than those collected on sugarcane ($F_{2,12}$ = 10.58; P = 0.006; 41.4 ± 6.9% and 29.7 ± 6.1%, respectively). In addition, metabarcoding detected bacteria (with abundances >1%) belonging to nineteen different genera in twelve orders (Supp Table 2 [online only]). A significantly greater proportion of *Arcobacter* sequences were detected in SCA associated with sugarcane than in SCA associated with sorghum ($F_{2,12}$ = 12.73; P < 0.01; Fig. 2, Supp Table 2 [online only]). In contrast, a greater proportion of *Citrobacter* sequences were detected in SCA associated with sorghum than in SCA associated with sugarcane ($F_{2,12}$ = 7.03; P = 0.02; Fig. 2, Supp Table 2 [online only]). Some aphid-associated bacteria occurred at a low abundance of reads and included *Acidovorax*, *Lactobacillus*, *Ralstonia*, *Rickettsiella*, and *Serratia* (Supp Table 2 [online only]). The sample from Louisiana sugarcane collected during 2007–2009 lacked *Citrobacter* and *Serratia*, while hosting *Rickettsiella* (Supp Table 3 [online only]).

Discussion

Advances in technology have allowed us to go from the identification of bacteria through classical microbiology techniques (Escobar-Zepeda et al. 2015) to the use of PCR to screen for specific bacterial taxa (Haynes et al. 2003, Vorburger et al. 2009, Ferrari et al. 2011, Brady et al. 2014) and now to the ability to screen for entire microbiomes using high throughput sequencing without any prior knowledge of their composition. Our approach using taxon-specific PCR and metabarcoding allowed us to screen for bacteria previously reported in aphids, while enabling us to identify other potential bacteria that SCA harbors.

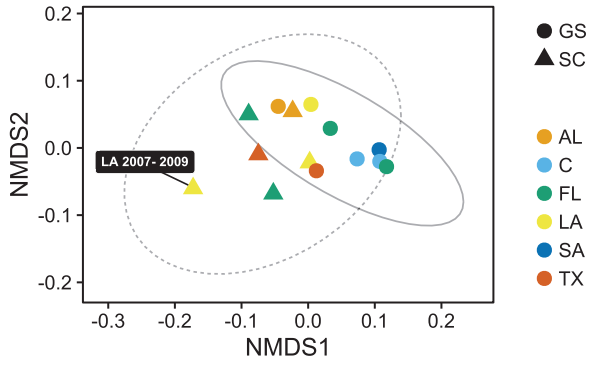


Fig. 1. NMDS (Non-Metric Multidimensional Scaling) analysis showing host-associated clustering in bacterial composition (PERMANOVA: $F_{1,12}$ = 4.73, P = 0.009). Samples collected before the pest outbreak, are marked with an a box labeled 2007–2009. The grain sorghum samples are surrounded by a solid ellipse while the sugarcane samples are surrounded by a dashed ellipse. Host plants: GS (grain sorghum) and SC (sugarcane). Locations: AL (Alabama), C (laboratory colony collected from Texas), FL (Florida), LA (Louisiana), SA (South Africa), and TX (Texas).

The use of PCR confirmed that sugarcane aphids harbor few commonly known aphid bacterial symbionts, with the exception of the obligate symbiont *Buchnera aphidicola* Buchner(Enterobacterales: Enterobacteriaceae) and facultative symbionts *Rickettsiella* and *Serratia* (identified in low abundances <1% with metabarcoding). Using metabarcoding we identified novel bacteria not previously reported in aphids (*Arcobacter*, *Bifidobacterium*, *Citrobacter*). In addition, metabarcoding allowed us to identify significantly higher abundances of bacteria in aphids from sugarcane (i.e., *Arcobacter*) when compared with aphids in grain sorghum (i.e., *Buchnera* and *Citrobacter*). A reduction in the abundance of the obligate symbiont *Buchnera aphidicola* in the presence of other bacteria is anticipated, as was shown with quantitative PCR in pea aphids that exhibited reduced *Buchnera* titers used to measure relative abundances in the presence of the secondary symbiont *Rickettsia* (Sakurai et al. 2005).

Bacterial symbionts can facilitate nitrogen use, breakdown sugar, and degrade pesticides in their insect hosts (Anderson et al. 2013, Ben-Yosef et al. 2014, Cheng et al. 2017). We identified *Bifidobacterium* in SCA collected from grain sorghum and sugarcane, a bacterium that it is known to break down carbohydrates in both insects and humans (Killer et al. 2009, O'Callaghan and van Sinderen 2016, Alberonia et al. 2019) and in SCA may play a role in processing phloem. In addition, two bacteria associated with detoxification in other insects were found. For example, *Citrobacter* is associated with increased insecticide resistance in the tephritid fruit fly *Bactrocera dorsalis* (Hendel) (Cheng et al. 2017). Similarly, bacteria in the genus *Pseudomonas* are known to detoxify caffeine in coffee berry beetles *Hypothemus hampei* (Ceja-Navarro et al. 2015) and may have a similar detoxifying potential in SCA.

Some bacteria may be biologically relevant even when found in low abundances (Stouthamer et al. 2018). While a relative abundance <1% may reflect some sequencing error, our use of stringent quality control filtering helped increase the reliability of low abundance reads (Bokulich et al. 2013). Both *Rickettsiella* spp. and *Serratia* spp. are reported to have biologically relevant functions at low abundances in other aphids (Enders and Miller 2016). Interestingly, *Rickettsiella*, which is known to alter body color in pea aphids (Tsuchida et al. 2010), was found in SCA and if similar in function, might influence the attractiveness of SCA to natural enemies (i.e., predators and parasitoids). SCA also harbored *Serratia*, which has been reported to provide heat tolerance in other aphids (Russell and Moran 2006, Oliver et al. 2010).

In addition to the potential symbionts mentioned above, we detected potential plant pathogens associated with members of the grass family (Poaceae) that include *Acidovorax* spp., *Corynebacterium* spp. and *Ralstonia* spp. The *Acidovorax* spp. group has been reported to cause tissue browning in some plants (Xie et al. 2011) and red stripe disease in infected sugarcane plants (Girard et al. 2014, Santa Brigida et al. 2016, Yonzone and Devi 2018). Similarly, *Corynebacterium* spp. is known as an animal and plant pathogen (Christie et al. 1991, Barba et al. 2015), with green peach aphid reported as capable of transmitting this bacterium to potatoes resulting in ring rot (Christie et al. 1991), whereas *Ralstonia* spp. is known to cause plant wilting and death in numerous agricultural crops (Álvarez et al. 2010, Meng 2013). Further investigation is required to determine whether the strains of these bacteria found in SCA are plant pathogens and if SCA is capable of their transmission.

While the bacteria reported above have been identified in other insects and some have known functions, other bacteria that we identified that have been reported from other insects require further investigation (i.e., *Acinetobacter*, *Arcobacter*,

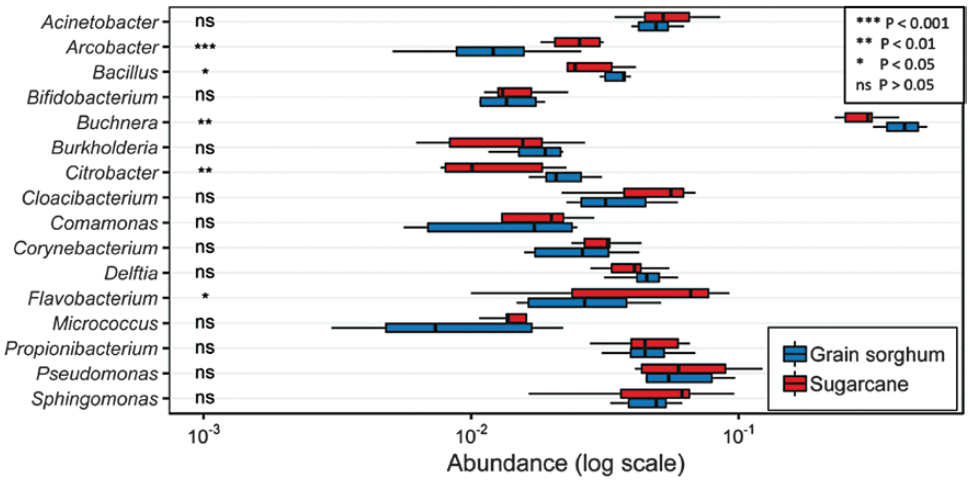


Fig. 2. Relative abundance of bacterial taxa in SCA collected from grain sorghum and sugarcane. Three bacterial genera (i.e., *Arcobacter, Buchnera,* and *Citrobacter*) showed significantly different abundances between aphids on different host plants. ns = $P > 0.05$; $*P = 0.02$; $**P < 0.01$.

Bacillus, *Burkholderia*, *Cloacibacterium, Delftia, Flavobacterium*, *Propionibacterium,* and *Sphingomonas;* Srivastava and Rouatt 1963, Kikuchi et al. 2005, Konig 2006, Xiang et al. 2006, Killer et al. 2009, Leroy et al. 2011, Malhotra et al. 2012, Morales-Jiménez et al. 2012, Cakici et al. 2014, Montagna et al. 2014, Ceja-Navarro et al. 2015, Meirelles et al. 2016, Meriweather et al. 2016, Segata et al. 2016, Cheng et al. 2017, Duguma et al. 2017, Luna et al. 2018). For example, *Flavobacterium* has been reported from pea aphids (Srivastava and Rouatt 1963), and while members of the order Flavobacteriales are reported to provision their insect hosts with nutrients (Wu et al. 2006, Bennett et al. 2014, Rosas-Pérez et al. 2014), it is unknown whether this bacteria would have a similar role in SCA. Similarly, *Micrococcus* in the European corn borer has potential contributions to gut enzymatic activity (Vilanova et al. 2012); however, its potential function in SCA is not known.

Interestingly, we did not detect bacterial symbionts that are well known from previous aphid studies with either PCR or metabarcoding. For example, we did not detect any reads associated with *Hamiltonella defensa,* which in another study was reported as a byproduct of bacterial read filtering from high-throughput sequencing of SCA (Harris-Shultz et al. 2017). We conclude that the presence of these other symbionts may be rare or completely absent in SCA populations in the United States.

We originally predicted that a change in the SCA microbiome composition might be responsible for SCA switching from sugarcane to grain sorghum resulting in the pest outbreak in 2013. While our results show host-associated bacterial differentiation in SCA collected from sugarcane and sorghum, these differences seem to correspond to the characterization of two genetically distinct aphid Multilocus Lineages or MLLs (Nibouche et al. 2018). These genetically distinct MLLs differ in host plant use: MLL-D prefers to feed on sugarcane and has likely been present in the United States since the 1970s while MLL-F prefers to feed on sorghum and was only recently detected in the United States (Nibouche et al. 2015, Nibouche et al. 2018). Therefore, the sudden SCA outbreak was likely caused by the introduction of a sorghum adapted strain of SCA, which also has host-associated bacterial differences, and not by the sudden acquisition or loss of a specific bacterial symbiont. Analysis of sugarcane aphids from Louisiana grain sorghum and sugarcane before and after the pest outbreak show an increase in the sorghum adapted MLL-F in sugarcane (Nibouche et al. 2018). In SCA, sugarcane populations had greater abundances of *Arcobacter* and a lower abundance of *Citrobacter* when compared to samples collected on sorghum. In fruit flies that harbor *Citrobacter* these bacteria enhance detoxification that enables resistance to insecticides (Cheng et al. 2017), which could have a similar function in SCA. The function of *Arcobacter* is still unknown despite its presence in multiple insect species. In addition, bacteria such as *Citrobacter* and *Serratia* (lacking in samples collected before SCA invasion on U.S. sorghum) were detected in aphids from both grain sorghum and sugarcane after 2013, suggesting that the sorghum adapted MLL-F aphids are spilling over into sugarcane. Alate aphids can be transported by wind to new locations and host plants (Wiktelius 1984, Irwin et al. 1988, Loxdale et al. 1993, Mann et al. 1995), which may explain why some SCA samples collected from sugarcane after 2013 (in Alabama, Florida, and Louisiana) have microbial compositions that cluster closer to SCA collected on grain sorghum. Our findings suggest a spillover of SCA from sorghum to sugarcane likely due to dispersal. Although changes in the microbiota composition between SCA before and after 2013 are unlikely to have caused the SCA outbreak in sorghum, the bacteria that we identified may play important roles in SCA.

Microbial organisms, such as bacteria, fungi, and viruses may play important roles in the management of insect pests (Lacey et al. 2001, Glare et al. 2012, Lacey et al. 2015). We currently know that some microorganisms influence the effectiveness of pest control strategies. For example, pea aphid association with specific strains of *Hamiltonella defensa* or *Regiella insecticola* confers increased resistance against parasitoid attacks (Oliver et al. 2005, Oliver et al. 2009, Vorburger et al. 2009), thus reducing the effectiveness of biological control. In contrast, pea aphids harboring some strains of *Serratia symbiotica* can be killed with lower doses of insecticide from different chemical classes including a neonicotinoid, carbamate, keto–enol, and organic thiophosphahate (Skaljac et al. 2018). Thus, in theory we could manipulate the microogranisms associated with insect pests, either by adding or eliminating microbes that benefit or harm pest control efforts. We currently use entomopathogens as biopesticides and manipulating symbiotic microorganisms would help expand pest control. The manipulation of pests' microbiotas as part of IPM may significantly reduce runoff and off-target effects associated with chemical control (Chandler et al. 2011, Dara 2017,

Lenteren et al. 2018) of some insect pests. The ability to incorporate relevant genes in insects and plants using novel gene editing methods (e.g., CRISPR-Cas9) provides another promising application for characterizing and manipulating pest insects' microbiota. Future approaches to genetic pest control may benefit not only from altering insect genes and their expression (e.g., through gene editing or RNAi for targeted gene silencing), but also by incorporating microorganisms' genes to enhance IPM.

Our research team plans to continue examining the role(s) that SCA bacteria may play in this insect pest. Future research will include assessing the prevalence of these bacteria among aphids in different locations as well as the potential detoxification role of *Citrobacter* in SCA. As was seen in pea aphids where their microbial composition influenced stress tolerance or fecundity (Oliver et al., 2010), these potential bacterial roles in SCA need to be characterized. In addition, screening of low abundance bacteria in different aphid populations, will allow us to determine transmission rates of bacteria in SCA. Last, further research into the potential role that microbes play in the attraction of natural enemies or mutualists to SCA is needed and could provide valuable insight for the use of biological control.

Overall, we found that populations of SCA in the continental United States contain only two of the well-studied facultative symbionts reported in aphids. It is important to note that HTS allowed us to identify bacteria in SCA, which had not been known to be associated with aphids, but have been hypothesized to act as potential symbionts in other insects. In addition, we found evidence of host-associated differentiation in SCA microbiota. This study provides a foundation for understanding the bacterial composition of SCA, which can be used to better inform Integrated Pest Management (IPM) of this pest in grain sorghum by providing novel targets for control.

Electronic Data Access

Raw data can be found on NCBI under accession number PRJNA578411. Scripts for data analysis are available through GitHub at: https://github.com/amalacrino/SCA_microbiota.

Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

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