Canadian Science Publishing | Genome

Next generation sequencing reveals endosymbiont variability in cassava whitefly, Bemisia tabaci, across the agro-ecological zones of Kerala, India

Journal:	*Genome*
Manuscript ID	gen-2018-0050.R3
Manuscript Type:	Article
Date Submitted by the Author:	20-May-2019
Complete List of Authors:	E. R., Harish; Central Tuber Crops Research Institute, Division of Entomology K., ManiChellappan; Kerala Agricultural University, Department of Entomology; KERALA AGRICULTURAL UNIVERSITY, AINPVPM T., Makeshkumar; Central Tuber Crops Research Institute, Division of Plant Pathology Mathew, Deepu; Kerala Agricultural University, Centre for Plant Biotechnology and Molecular Biology M. T., Ranjith; Kerala Agricultural University, Department of Agrl. Entomology D., Girija; Kerala Agricultural University, Department of Agrl. Microbiology
Keyword:	Arsenophonus, cassava mosaic, NGS, 16S rDNA, symbiotic bacteria
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

Next-generation sequencing reveals endosymbiont variability in cassava whitefly, *Bemisia tabaci,* across the agro-ecological zones of Kerala, India

E.R. Harish [1*], ManiChellappan[2], T. MakeshKumar[1], Deepu Mathew[3], M.T. Ranjith [2] and D. Girija[4]

[1]ICAR-Central Tuber Crops Research Institute, Sreekaryam, Thiruvananthapuram-695 017, India

[2]Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Thrissur – 680 656, India

[3]Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University, Thrissur – 680 656, India

[4]Department of Agricultural Microbiology, College of Horticulture, Kerala Agricultural University, Thrissur – 680 656, India

*For correspondence: E-mail: harisher4@gmail.com

ABSTRACT

Silverleaf whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most notorious invasive insect pests reported, infesting more than 900 species of plants and spreading more than 200 viral diseases. This polyphagous agricultural pest harbours diverse bacterial communities in its gut, which perform multiple functions in whiteflies, including nutrient provisioning, amino acid biosynthesis and virus transmission. The present exploratory study compares bacterial communities associated with silverleaf whitefly infesting cassava, also known as cassava whitefly, collected from two different zones (zone P: plains, zone H: high ranges), from Kerala, India, using next-generation sequencing of 16S rDNA. The data sets for these two regions consisted of 1,321,906 and 690,661 high quality paired end sequences with mean length of 150 bp. Highly diverse bacterial communities were present in the sample containing approximately 3,513 operational taxonomic units (OTUs). Sequence analysis had shown a marked difference in the relative abundance of bacteria in the populations. A total of 16 bacterial phyla, 27 classes, 56 orders, 91 families, 236 genera and 409 species were identified from P population, against 16, 31, 60, 88, 225 and 355, respectively in H population. *Arsenophonus* sp. (Enterobacteriaceae), which is important for virus transmission by whiteflies, was relatively abundant in P population, whereas in H population *Bacillus* sp. was the most dominant group. The association of whitefly biotypes and secondary symbionts suggests a possible contribution of these bacteria to host characteristics such as virus transmission, host range, insecticide resistance and speciation.

Keywords: *Arsenophonus;* cassava mosaic; NGS; 16S rDNA; symbiotic bacteria

Introduction

Microorganisms and insects are the two most successful groups of organisms on planet earth. The ubiquitous microbes are also present inside the body of insects in close association and are known as endosymbionts. Of the symbionts, bacteria perform the most diverse roles and hence most studied (Engel and Moran 2013). Less than 1% of endosymbionts are culturable, but techniques such as next-generation sequencing enable researchers to explore more deeply the true complexity of insect-microbe associations and to understand the profound influence of microbial metabolic activity on the other organisms (Schloss and Handelsman 2003).

Endosymbiotic theory (Mereschkowski 1910) classifies endosymbionts into primary and secondary. Primary endosymbionts (P-endosymbionts) shall be associated with insect hosts for long period of time and form obligate associations and co-speciation with their insect hosts, whereas secondary endosymbionts (S-endosymbionts) are more recently developed associates which sometimes get horizontally transferred between hosts, live in the hemolymph of the insects but never obligate. S-symbionts are not confined into specialized S-bacteriocytes found in gut tissues, glands, body fluids, cells surrounding the P-bacteriocytes or even invading the P-bacteriocytes themselves (Baumann et al. 2006). S-symbionts seem to be the result of multiple independent infections and, although they are usually maternally inherited, their transmission may also occur horizontally across the hosts.

These microbes help in a variety of ways for the dominance of insects. They provide various fitness advantages, such as increased fecundity, increased longevity, female-biased sex ratio as well as greater immunity against natural enemies and pathogens (Su et al. 2013). S-symbionts are shown to increase resistance of insects to fungal pathogens (Aksoy et al. 1997). In their host insects, endosymbionts play various roles such as nutrition; eg. *Buchnera* provides essential amino acids that are lacking in the plant sap diet of its aphid host (Xie et al.

2018), detoxification of toxins, plant allelochemicals and insecticides (Kikuchi et al. 2012), source of cues and signals (Dillon et al. 2002), defense toward pathogens and parasites (Oliver et al. 2003), adaptation to environment shock (Montllor et al. 2002), virus-vector interaction (Chiel et al. 2007), population dynamics (Kikuchi et al. 2012), insect-plant interactions (Hosokawa et al. 2007), biotype determinants (Gueguen et al. 2010) and protectants against natural enemies (Oliver et al. 2003). Rosell et al. (2010) described mutualistic and dependent relationships of endosymbionts with other organisms.

Sequencing the 16S ribosomal RNA (rRNA) was the most popular method adopted to identify the bacteria (Petti et al. 2005; Ranjith et al. 2016). Nearly 1500 bp of 16S rDNA is large enough for bioinformatics analyses (Patel 2001) and additionally, it is present in all bacteria and function is well defined (Janda and Abbott 2007). But, this fails in polymicrobial specimen wherein multiple templates result in uninterpretable Sanger reads (Drancourt 2000). Next-generation sequencing with the primer spanning hypervariable regions (V1-V9) of 16S rRNA gene circumvents these limitations. Metagenomics applies a suite of genomic technologies and bioinformatics tools to directly access the genetic content of entire communities of organisms (Thomas et al. 2012).

Root and tuber crops, including cassava, provide a substantial part of the world's food supply (Chandrasekara and Kumar 2016). Cassava whitefly (*Bemisia tabaci*) transmitted cassava mosaic disease causes 30-40% yield losses on a global scale (Malathi et al. 1985). As a phloem-feeding homopteran, *B. tabaci* harbours various endosymbionts for its nutritional requirements and functionality. These endosymbionts act as carotenoids sources of whiteflies (Sloan and Moran 2012); few endosymbionts such as *Portiera aleyrodidarum* provides B-complex vitamins and amino acids lacking in fly feeds (Lai et al. 1996; Xie et al. 2018). They also assist in virus transmission (Nirgianaki et al. 2003; Chiel et al. 2007) and a GroEL homologue from endosymbionts assists in the circulative transmission of virus by protecting

the virus from destruction during its passage through the haemolymph (Morin et al. 1999; Rana et al. 2012). According to Gottlieb et al. (2010), *Hamiltonella* sp. found in B-biotype of whiteflies help them to become successful vectors of *Tomato yellow leaf curl virus*.

Endosymbionts play crucial roles in making whitefly a pest of global importance. Keeping in view of the economic losses by cassava whitefly, as the vector of *Cassava mosaic virus*, this study answers the hypothesis that under different agro-ecological zones, at various disease severities and in genetically varying populations, the endosymbiont population varies. Using next-generation sequencing of 16S rDNA from gut microbiome, variation in the relative abundance of different bacterial endosymbionts in cassava whitefly populations from different agro-ecological zones of Kerala, India, is shown. Further, the pest management application of the understanding of endosymbiont variability under specific physiological conditions is also discussed.

Materials and methods

Collection of whiteflies

Bemisia tabaci samples were collected from cassava fields of 13 different agro-ecological zones of Kerala (Mohankumar 2007) (Latitudes 8.1730° N to 12. 4740° N and Longitudes 74.2747° E to 77.3712° E), India (Harish et al. 2016; Supplementary Table 1; Supplementary Figure 1). Whiteflies were collected individually (March 2014-May 2016) in microfuge tubes containing 70% ethanol and stored at -20 °C. Each sample was identified as *B. tabaci* by adopting the morphological identification keys (Martin 1987).

Genetic variability study/ barcoding using mitochondrial *Cytochrome oxidase1* gene (primers C1-J2195 and L2-N-3014) had shown the presence of two biotypes/ genetic groups of *Bemisia*, Asia I and Asia II5 in cassava plants of Kerala and the biotypes were confirmed by using reference sequences from NCBI database. According to Dinsdale et al. (2010), 3.5% genetic divergence is the minimum requirement to separate two putative species/ biotypes/

genetic groups of whitefly. In this case, up to 15.96% sequence divergence was observed between the biotypes. Whiteflies collected from plains (elevation less than 150 m from MSL) belonged to Asia II5 biotype, whereas whiteflies collected from hilly regions (elevation more than 900 m) belonged to Asia I biotype. Composite samples, P and H, were made from the two biotypes identified (biotypes Asia II5 from plains and Asia I from hills) and analysed using metagenomic approach to compare the endosymbiont variations. Sample P included one whitefly each from 12 agro-ecological zones of plains (<150 m from MSL) and H had 12 whiteflies from hilly regions of Sulthan Bathery. Also, from the cassava plants surveyed for severity of cassava mosaic disease symptoms, plants in high elevations of Sulthan Bathery region (> 900 m from MSL) had shown very less severity with a score of 1 compared to plants from other areas (severity score 3-5, Ikotun and Hahn 1994).

Isolation of metagenomic DNA from adult *Bemisia tabaci*

The insects were surface sterilized with ethanol and household chlorine bleach, as described by Davidson et al. (1994). Metagenomic DNA from samples P and H were isolated through the direct method (Zhou et al. 1996). They were homogenized in 400 µl of extraction buffer [200 mM Tris-HCl (pH-8.0), 25 mM EDTA (pH-8.0), and 250 mM NaCl, SDS (0.5%)] in a 1.5 ml Eppendorf tube, using liquid nitrogen. The homogenized samples were incubated at room temperature for 1 hr and centrifuged at 12,000 rpm for 5 min. at 4 ºC. The supernatants were collected in fresh tubes, and equal volume of phenol:chloroform:isoamyl alcohol (24:25:1) was added to the supernatants and again centrifuged at 10,000 rpm for 20 min. at 4 °C. The aqueous phase was transferred to fresh tube and equal volume of iso propanol was added, the mixture was incubated at room temperature for 15 min., centrifuged at 13,000 rpm for 5 min. at room temperature and the metagenomic DNA pellet was precipitated out. DNA pellet was washed with ethanol (95%) by centrifugation at 10,000 rpm

for 10 min., air dried, dissolved in 25 µl of autoclaved distilled water and stored in deep freezer (-80 °C) for future use.

Quality checking of metagenomic DNA

The 16S rDNA fragment was amplified by polymerase chain reaction from the metagenomic DNA using the universal 16S rDNA primers fD1 (5'-GAGTTTGATCCTGGCTCAG-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') (Haris et al., 2014). Reaction had 0.2 µl template DNA (20 ng), 0.1 µl each of the forward and reverse primers, 1 µl of 10 mM dNTP (Genei®), 0.2 µl of Taq DNA polymerase (Genei®), 2.5 µl of Taq DNA buffer A (Genei®) and 15.9 µl of grade I water. Thermal cycling included initial denaturation at 94 °C for 2 min. followed by 29 cycles with denaturation at 94 °C for 45 sec., primer annealing at 55 °C for 1 min. and primer extension at 72 °C for 2 min. and final extension at 72 °C for 10 min. The products were electrophoresed on agarose gel (0.8%).

Sequencing of 16S ribosomal RNA amplicon

The metagenomic DNA isolated from *B. tabaci* adults were sequenced using Next-Generation Illumina MiSeq™. Amplicon library was prepared with specific primers spanning the hypervariable V3 region of 16S rRNA gene (Supplementary Figure 2) and used for sequencing and subsequent classification.

Amplicon PCR

Metagenomic DNA samples were normalized to 5 ng/ µl in 10 mM Tris (pH 8.5) and amplicon PCR was carried out using V3 primers (341F - 5'CCTACGGGAGGCAGCAG3', 518R - 5'ATTACCGCGGCTGCTGG3') (Bartram et al. 2011). PCR master mix consisted of 2 µl each of forward and reverse primers (10 pM/ µl), 0.5 µl 40 mM dNTPs, 5 µl 5X Phusion HF reaction buffer, 0.2 µl 2U/ ul or µl F-540 Special Phusion HS DNA polymerase, 5 ng input DNA and water to make up the volume to 25 µl. PCR reaction was programmed with

initial denaturation at 98 °C for 30 sec. followed by 30 cycles of denaturation at 98 °C for 10 sec., primer annealing at 55 °C for 30 sec., primer extension at 72 °C for 30 sec. and final extension at 72 °C for 5 min. PCR products were quantified using the fluorescence quantitative fluorometer (Qubit 2.0®) with the Qubit dsDNA HS assay kit (Invitrogen, USA).

16S rDNA amplicon library preparation

PCR clean-up

PCR clean-up was carried out using AMPure XP beads to purify the 16S V3 amplicon away from free primers and primer dimer species. The reagents consisted of 10 mM Tris (pH 8.5) (52.5 µl per sample), AMPure XP beads (20 µl per sample) and freshly prepared ethanol (80%) (400 µl per sample). Standard protocol (Amplicon et al. 2013) was followed and the cleaned products were stored at -20 °C.

Index PCR

Illumina™ Truseq adapters and indices were added to the cleaned PCR products. PCR master mix consisted of 2 µl each of 10 pM/ µl forward and reverses primers, 1.0 µl 40 mM dNTP, 10 µl 5X Phusion HF reaction buffers, 0.4 µl 2U/ µl F-540 special Phusion HS DNA polymerase, 10 µl (minimum 5 ng) PCR1 amplicon and water to make up the total volume to 50 µl. PCR reaction was programmed with initial denaturation at 98 °C for 30 sec., 15 cycles with denaturation at 98 °C for 10 sec., primer annealing at 55 °C for 30 sec. and primer extension at 72 °C for 30 sec. followed by final extension at 72 °C for 5 min.

PCR clean-up 2

AMPure XP beads were used to clean up the final library before quantification. The reagents consisted of 10 mM Tris (pH 8.5) (27.5 µl per sample), AMPure XP beads (56 µl per sample), freshly prepared 80% ethanol (400 µl per sample).

Library quantification, normalization, and pooling

Libraries were quantified using a fluorometric quantification method and concentrated final library was diluted using distilled water. Diluted DNA (5 µl) from each library was pooled with unique indices.

Library denaturing and MiSeq sample loading

In preparation for cluster generation and sequencing, pooled libraries were denatured with NaOH, diluted with hybridization buffer, and then heat denatured before MiSeq® sequencing. Each run included a minimum of PhiX (5%) to serve as an internal control for these low diversity libraries. Denatured library was loaded into the reagent cartridge of Illumina MiSeq™ sequencer. The output files (fastq) generated from the sequencer were analysed.

Analysis of NGS data

Total raw reads of samples obtained from Illumina sequencing platform were quality checked for base quality (Phred Score), base composition, adapter dimer contamination, ambiguous bases and GC content using Fast QC (Version0.11.8) tool with default parameter. The 16S rDNA V3 hypervariable region specific primers were checked in the paired-end reads and allowed to merge using Clustal Omega (version 1.2.0) program with minimum overlap length of 10 bp. The merged consensus fasta of all samples were pooled and taken for various downstream analyses.

As a part of pre-processing of sequence reads, singletons, generated due to the sequencing errors and could result in spurious operational taxonomic units (OTUs), were removed before starting OTU clustering, by removing the reads that did not cluster with other sequences (abundances <2). Singleton are the R1+R2 merged consensus of V3 FASTA contig sequence whose frequency is only one, or present only one time. Chimeras were also removed using the *de novo* chimera removal method UCHIME implemented in the tool USEARCH.

Using Uclust program, pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity (similarity cut off at 0.97). QIIME (Caporaso et al. 2010) and MG-RAST (Meyer et al. 2008) programmes were used in downstream analyses. Representative sequences were identified for each OTU and aligned against Greengenes core set of sequences using PyNAST program (DeSantis et al. 2006a, 2006b). Further, these representative sequences were aligned against reference chimeric datasets, and taxonomic classification was performed using RDP classifier and Greengenes OTUs database.

The taxonomic categories of bacteria (from phylum to species level) present in both P and H populations of *Bemisia tabaci* were compared using Jaccard distance (a measure of how dissimilar the sets are) in R software (version 3.6.0). More the distance between each taxonomic category means more the variation between them.

Community matrices at phylum, class, order, family, genus and species levels were prepared for both the populations and the relative abundances (Pi) were prepared based on OTU counts. The data was further analyzed using Shannon Weiner diversity Index, with function $H = \sum[(Pi) \times \ln(Pi)]$. Pi is per cent of a particular bacterium compared to the total bacteria identified in that population at that taxonomic level, using the pre-processed total reads. For example, when we compare the variability between P and H populations at Phylum level in terms of the bacterium Proteobacteria, P population has the per cent abundance of 87.57 whereas it is 13.40 in H population. Thus, Pi values shall be 0.8757 and 0.1340, respectively and corresponding index values -0.116 (0.8757*-0.1327) and -0.269 (0.1340*-2.0099), respectively. Index ratio between the populations indicated the variation across both the populations in each level. Similarly, at each level of taxonomic classification, indices for every bacterial community were calculated and compared.

Rarefaction analysis was carried out to assess species richness of the samples based on the construction of rarefaction curves using MG-RAST software. A phylogenetic tree of bacteria at family level was also constructed using MG-RAST with Illumina sequencing data set. The RDP database was used as annotation source, and a minimum identity cutoff (90%) was applied.

Sequence Read Archive (SRA) submission

Metagenomic sequences were submitted to Sequence Read Archive (SRA) at https://submit.ncbi.nlm.nih.gov/subs/sra/. Experiment ID and Run ID were received for each submission.

Results

Isolation and quality checking of metagenomic DNA from adult *Bemisia tabaci*

Metagenomic DNA isolated from the two samples of *B. tabaci* were confirmed with the presence of 16S rDNA fragment in the isolated products by amplification of band of 1500 bp with universal 16S rDNA primers (Supplementary Figure 3). The metagenomic DNA was quantified with fluorometer (Qubit 2.0) and the concentrations were 30.6 ng/ µl and 30.4 ng/ µl, respectively, in samples H and P. The hypervariable V3 region of 16S rDNA was amplified with specific primers (Supplementary Figures 4a, 4b) and preceded with 16S rDNA library preparations.

Illumina sequencing data

Total raw sequencing reads (paired end) of 1,321,906 and 690,661 with average sequence length of 150 bp each was obtained from Illumina MiSeq™ sequencer. The quality of left and right end of the paired-end read sequences of the sample are shown in the Supplementary Figures 5a, 5b and 6a, 6b. Nearly 90% of the total reads had phred score greater than 30 (>Q30; error-probability <= 0.001).

The base composition distribution of two samples was adenine (24.05%, 23.63%), cytosine (24.06%, 24.64%), guanine (27.46%, 27.78%) and thiamine (24.43%, 23.95%) and the average GC content was 40-50%. Application of multiple filters such as conserved region filter, spacer filter, quality filter and mismatch filter had resulted 1240613, 1240116, 1239993 and 640996 reads, respectively for sample P. For H, corresponding values were 640923, 640500, 640450 and 341937. While making consensus V3 sequence, more than 48% of the paired-end reads were aligned to each other with zero mismatches with an average contig length of 135 to 165 bp (Supplementary Figures 7a and 7b).

From the 640,996 and 341,937 consensus reads from samples, singletons and chimeric sequences were removed to obtain 611,218 and 334,634 high quality pre-processed reads. These were pooled and clustered into OTUs based on their sequence similarity (similarity cut off = 0.97) and a total of 3,513 OTUs were identified from 945,852 reads (Figure 1).

The rarefaction analysis, carried out to verify the amount of sequencing reflected in the diversity of original microbial community, has revealed that the slopes of the curves decline markedly with increasing sequences (Figure 2). The alpha diversity (6.22 and 3.82 for P and H populations, respectively) indicated the extent of bacterial species diversity present in *B. tabaci*.

Composition of bacterial community of *Bemisia tabaci*

The bacteria present in adult *B. tabaci* were analysed and taxonomically grouped from phyla to species levels using RDP classifier and Greengenes OTUs database. The relative abundance of 10 major bacterial groups in each taxonomic category is given in Tables 1 and 2. Altogether, 16 bacterial phyla were detected from samples P and H. Most dominant phylum in P population was *Proteobacteria* (87.5% of total bacterial community) and in H

population, it was *Firmicutes* (82.6%). This was followed by *Firmicutes* (9.3%) in P population and *Proteobacteria* (13.4%) in H population.

P population had *Bacteroidetes* bacteria to the tune of 2.9%. *Chlorobi, Actinobacteria, Planctomycetes, Verrucomicrobia, Spirochaetae, Tenericutes* and *Acidobacteria* were other phyla, constituting less than 1% whereas, H population had only a meagre count of *Bacteroidetes*, *Chlorobi, Actinobacteria, Planctomycetes, Verrucomicrobia, Spirochaetae,Tenericutes* and *Acidobacteria.* A total of 27 and 31 bacterial classes were identified for P and H population, respectively. For sample P, class *Gammaproteobacteria* was most dominant (86.4%) and for H, it was *Bacilli* (82.6%). In population P, 56 bacterial orders have been detected of which *Enterobacteriales* was dominant (85%). Of the 60 orders seen in population H, *Bacillales* was most dominant (82.5%). Analyses at family level have revealed a total of 91 and 88 bacterial families in P and H population, respectively, major groups being *Enterobacteriaceae*, *Bacillaceae*, *Flavobacteriaceae*, *Vibrionaceae*, *Oxalobacteraceae etc*. for P and *Enterobacteriaceae*, *Bacillaceae*, *Alcanivoracaceae etc*. for H (Figure 3). Among the 236 genera identified in P sample, *Bacillus* (35.5%) and *Arsenophonus* (24.6%) were the most dominant. Other important genera in P population were *Vibrio*, *Riemerella*, *Lysinibacillus*, *Flavobacterium*, *Janthinobacterium*, *Sphingobacterium*, *Bacteroides* and *Enterococcus*. For H population, the order of abundance was *Bacillus* followed by *Alcanivorax*, *Staphylococcus*, *Pantoea*, *Lysinibacillus*, *Bacteroides*, *Alistipes*, *Photorhabdus*, *Terribacillus* and *Enterococcus*. A total of 409 species were identified in sample P and 355 in sample H (Figure 2). From RDP database, sequence similarities were observed with *Arsenophonus* spp., *Bacillus* spp., *Riemerella anatipestifer*, *Vibrio harveyi*, *Lysinibacillus sphaericus* and *Janthinobacterium* spp. for P population and *Bacillus thuringiensis*, *Staphylococcus* spp. *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Pantoea dispersa* and *Bacillus pumilus* for H population.

Comparison between different taxonomic categories of bacteria present in P and H populations of *Bemisia tabaci* using Jaccard dissimilarity index shows that at species level, the bacteria present in both P and H populations of *B. tabaci* showed maximum dissimilarity to the tune of 99.9%, whereas the bacteria at genus level were recorded the least dissimilarity of 50.5%. At phylum level, the bacteria were dissimilar to the tune of 84.9%, whereas 82.7% of dissimilarity at class level was observed among bacteria population in P and H populations of *B. tabaci*. The bacteria at order and family level were dissimilar to the tune of 88.8-88.9%. Shannon Weiner diversity index was used to assess the relative diversity of bacteria in both the populations at each taxonomic level (Supplementary Table 4). Higher index values had shown higher diversity of the bacterium. At phylum, class and order levels, both the populations were found to have equal level of diversity, though they differed in four bacterial orders. At family level, population H was distinctly more diverse but at genus level population P had shown better diversity. This shows that different genus found in population P belonged to same family and even though the number of genera was less, bacteria in population H belonged to different family. Diversity index at species level was high (> 1.0) in both the populations and the species accommodated in both populations varied.

SRA submission: SRA submission has generated the experiment ID SRX1592694 and run ID SRR3178391.

Discussion

Microbial community vary between our study populations

Polyphagous agricultural pests harbour diverse bacterial communities in their gut, which assist diverse functions including polyphagy and general fitness. In the present study, bacterial communities associated with cassava whiteflies collected from different agro-ecological zones of Kerala, India were compared. Metagenomic DNA of *B. tabaci* strains have been isolated by standard protocol (Zhou et al. 1996) and sequenced in Illumina NGS

platform. Analysis of hypervariable V3 region of 16S rDNA fragment resulted in 1,321,906 and 690,661 high quality paired end sequences with mean length of 150 bp. Number of bacterial species detected was a function of the number of sequence analyzed (Shi et al. 2012). Highly diverse bacterial communities were present in the sample containing approximately 3,513 operational taxonomic units (OTUs). Studies by Chiel et al. (2007) and Gueguen et al. (2010) for identifying bacterial community of *B. tabaci* also have used the amplification of 16S rDNA of bacteria. Parallel studies on *B. tabaci* from 14 different locations in Northern India, using 16S rDNA clone library sequences had shown that *Portiera* is the primary endosymbiont and secondary endosymbionts include *Cardinium*, *Wolbachia*, *Rickettsia* and *Arsenophonus* along with *Bacillus, Enterobacter, Paracoccus* and *Acinetobacter* (Singh et al. 2012) but in the present study *Portiera* was not identified, whereas secondary endosymbionts such as *Bacillus, Arsenophonus, Enterococcus, Bacteroides* etc. were identified.

Downstream analysis using QIIME (Caporaso et al. 2010) and MG-RAST (Meyer et al. 2008) and statistical analysis using Shannon Weiner diversity index (Shannon and Weaver 1949) had shown a marked difference in relative diversity of bacteria in the populations at various taxonomic levels. Altogether, 16 bacterial phyla were detected from P and H samples. Among the phyla from P population, *Proteobacteria* was most dominant followed by *Firmicutes* and *Bacteroidetes* and for H population, it was *Firmicutes*, *Proteobacteria* and *Bacteroidetes*. Su et al. (2016), identified 27 different phyla of bacterial community associated with *B. tabaci*, from different crops, in which; *Proteobacteria* (94.0-98.0%) was the most dominant, followed by *Bacteroidetes* (0.5-4.5%) and *Firmicutes* (0.2-2.0%) and the present study also shown similar results.

Importance of endosymbionts for insect function

Bacterial endosymbionts are essential for survival, spread and evolution of *B. tabaci*

(Thao and Baumann 2004; Himler et al. 2011). Even though they are known to perform a variety of roles in whiteflies (Rana et al. 2012; Xie et al. 2018), functions of many of these endosymbionts remain still unknown. Detailed examination of their presence and functions in other insects with the help of literature can provide an idea about their possible roles in whiteflies (Supplementary Tables 2 and 3). *Proteobacteria* associated with insects aid in carbohydrate degradation (Delalibera et al. 2005), synthesis of B vitamins and essential amino acids (Bennet et al. 2014) and pesticide detoxification (Werren 2012). Oesi-Poku et al. (2012) and Jones et al. (2013) found that *Proteobacteria* is typically the predominant bacterial taxon in the gut of mosquitoes. *Proteobacteria* followed by *Firmicutes* and *Actinobacteria* were the major bacterial phyla detected in the midgut of *H. armigera* larvae (Priya et al. 2012), gut and reproductive organs of both male and female fruit fly *Bactrocera minax*, gut of ground beetles (Jonathan et al. 2007), and desert locust, *Schistocerca gregaria* (Dillon et al. 2010). However, *Bacteriodetes* and *Firmicutes* were dominant in gut of termites (Xiang et al. 2012) and bees (Mohr and Tebbe 2006). Some members of *Firmicutes* assist insects in cellulose and hemicellulose digestion (Brown et al. 2012). Higher termites harbour *Bacteroidetes*, in their hindgut, to degrade lignocellulose, with the host enzymes acting on the amorphous regions of cellulose and the symbiotic enzymes targeting the crystalline regions (Brune 2014). These bacteria also induce cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella* (Hunter et al. 2003).

In the present study on whiteflies, for both P and H populations, reads for phyla *Chlorobi, Actinobacteria, Planctomycetes, Verrucomicrobia, Spirochaetes, Tenericutes* and *Acidobacteria* were also seen. *Chlorobi* is a salivary-associated unique bacterial community in *Anopheles culicifacies* (Sharma et al. 2014), and their role in the insect is unknown. In insects, *Actinobacteria* exhibit diverse physiological and metabolic properties such as production of extracellular enzymes and formation of a wide variety of secondary metabolites

(Schrempf 2001). In termites, they assist in nutrient acquisition from polysaccharides including cellulose (Pasti and Belli 1985; Watanabe et al. 2003) and hemicellulose (Schafer et al. 1996). According to Douglas (2015), *Actinobacteria* is the most dominant phylum of bacteria in whitefly, followed by *Bacteroidetes*, *Firmicutes*, and *Proteobacteria.* This variation can be attributed to the host variations.

The extreme alkalinity in some compartments of termite guts supports the growth of specialized alkaline-tolerant symbiotic bacteria from *Planctomycetes* (Köhler et al. 2008; Bignell 2010). Beetles and termites feeding on wood or detritus have higher populations of *Verrucomicrobia* in their gut (Colman et al. 2012), and in the hindgut of the wood-feeding termites, *Spirochaetae* are present in abundance (Köhler et al. 2012). The *Tenericutes* are present in termites and cockroaches also (Sabree and Moran 2014). *Acidobacteria* were identified from the larvae of the cerambycid *Leptura rubra* feeding on rotten softwood (Grünwald et al. 2010). *Acidobacteria* uses plant polymers, including xylan and cellulose, and degrade these polymers in the larval gut (Eichorst et al. 2011).

Using 16S rDNA clone library sequences, Singh et al. (2012) identified more than 300 bacterial genera from whiteflies, including secondary endosymbionts such as *Cardinium*, *Wolbachia*, *Rickettsia* and *Arsenophonus*, *Bacillus*, *Enterobacter*, *Paracoccus* and *Acinetobacter.* Secondary endosymbionts were not uniformly distributed in different locations. In the present study, 236 and 225 bacterial genera were present in P and H population, respectively. For P population, *Bacillus* was the most dominant group followed by *Arsenophonus*, *Vibrio*, *Riemerella*, *Lysinibacillus*, *Flavobacterium*, *Janthinobacterium*, *Sphingobacterium*, *Bacteroides*, *Enterococcus* and for H population, the order of relative abundance was *Bacillus*, *Alcanivorax*, *Staphylococcus*, *Pantoea*, *Lysinibacillus*, *Bacteroides*, *Alistipes*, *Photobacterium*, *Terribacillus* and *Enterococcus*. At species level, a total of 409 species were identified in sample P and total of 355 species were identified in sample H.

Secondary endosymbiont of *Bemisia tabaci* [unspecified/ unidentified by the software and identified as a single species group], *Arsenophonus*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus flexus*, *Riemerella anatipestifer*, *Vibrio harveyi*, *Lysinibacillus sphaericus*, *Janthinobacterium* sp. and *Bacillus pumilus* were the major 10 species identified for P population. For H population, the major species identified were *Bacillus thuringiensis*, *Alcanivorax* sp., *SBR proteobacterium*, *Staphylococcus pasteuri*, *Bacillus amyloliquefaciens*, *Staphylococcus sciuri*, *Bacillus megaterium, Pantoea dispersa*, *Lysinibacillus sphaericus* and *Bacillus pumilus*.

Many *Bacillus* members are present in *B. tabaci* and they may contribute in nutrition. According to Chandler et al. (2011), host diet has a greater effect on the bacterial microbiome composition. As *Bacillus* strains have the ability to produce amylase enzyme (Amund and Ogunsina 1987; Oyewole and Odunfa 1992), these amylases may be involved in the initial breakdown of cassava starch into simple sugars. *Bacillus megaterium* isolates were found to produce medium-length sugars from sucrose (Davidson et al. 1994). Also, *Bacillus* spp. associated with *B. tabaci* may produce long-chain sugars which contribute to the stickiness of the honeydew of the insect (Davidson et al. 1994).

Interestingly, *Bemisia* also harbours various entomopathogens such as *Bacillus thuringiensis* (Raymond et al. 2010; Walters et al. 1995) and *Bacillus cereus* (Song et al. 2014), which are effective biocontrol agents for whiteflies (El-Assal et al. 2013). *Bacillus pumilus* is effective in reducing second nymphal instar populations of *B. tabaci* (Ateyyat et al. 2010) and entomopathogen *Bacillus megaterium* manages *Aphis pomi* (Aksoy and Ozman-Sullivan 2008). *Bacillus flexus* induces the oviposition of sand fly (*Phlebotomus papatasi*) (Mukhopadhyay et al. 2012), whereas *Bacillus amyloliquefaciens* has strong mosquito larvicidal and pupicidal action, and are used in mosquito control programmes (Geetha et al. 2014).

Endosymbiont, *Arsenophonus* is important in virus transmission by whiteflies (Rana et al. 2012) and are relatively abundant in P population. GroEL molecular chaperones from *Arsenophonus* sp. are found to be associated with coat proteins of *Cassava mosaic virus* and help them from disintegration in the insect haemolymph. Similar results are reported by Morin et al. (1999) and Gottlieb et al. (2010) in the case of *TYLCV* (*Tomato yellow leaf curl virus*), for *Buchnera* GroEL and *Hamiltonella* GroEL respectively. Since cassava plants from where P population of whiteflies are collected had shown high severity (Scale 3-5) (Ikotun and Hahn 1994) of cassava mosaic disease, the results are in agreement with the findings of Rana et al. (2012). Our results indicate a possible association of whitefly endosymbiont, *Arsenophonus* with *Cassava mosaic virus* in its transmission. Compared to disease spread by P population, cassava mosaic disease intensity was negligible (Scale 0-1) in Sulthan Bathery, where *Arsenophonus* was absent in the H population. *Arsenophonus* is also suspected to reduce the fecundity of its host (Gherna et al. 1991; Duron et al. 2008). Raina et al. (2015) observed that the elimination of *Arsenophonus* and decrease in the diversity of bacterial symbionts by antibiotic treatment leads to increase in fitness of whiteflies.

Luciferases from luminous bacteria, *Vibrio harveyii* and the presence of *Riemerella*, in ant species, *Nylanderia fulva* were reported by Schmidt et al. (1989) and McDonald (2012), respectively. Santos-Garcia et al. (2014) reported symbiotic association of *Alcanivorax* in moss bugs to fulfil their nutritional requirements, resulting from their unbalanced diet and their role in marine oil-spill degradation is reported by McGenity et al. (2012). Apart from whitefly management, *Lysinibacillus sphaericus* (El-Assal et al. 2013) can also be used as a biological control agent for insecticide-resistant *Aedes aegypti* (Rojas-Pinzón and Dussán 2017). *Staphylococcus* from *Bemisia* produces medium length sugars from sucrose and contributes to the stickiness of the honeydew secreted by the host insect (Indiragandhi et al. 2010). McDonald (2012) reported the presence of *Staphylococcus*

456 *pasteuri* and *Staphylococcus sciuri* from ant species *Nylanderia fulva* and Ateyyat et al.
457 (2010) reported their potential as biocontrol agents.

458 Rosenblueth et al. (2012) reported evolutionary relationships of flavobacterial
459 endosymbionts with their scale insect hosts. The endosymbiont *Pantoea*, observed in the
460 study may perform semiochemical effects, as it is already reported for *Pantoea agglomerans*,
461 which is producing a chemical Guaiacol and helps in the aggregation of desert locust,
462 *Locusta migratoria* (Dillon and Charnley 2002; Davis et al. 2013). *Pantoea dispersa* is
463 reported in wild mosquito *Aedes albopictus* (Moro et al. 2013). Evidence for the microbial
464 utilization of nitrogenous waste products by *Bacteroides* has been obtained for termites,
465 cockroaches and hemipterans (Potrikus and Breznak 1981). *Janthinobacterium* strains
466 reported to have the capacity to degrade chitin (Gleave et al. 1995; Xiao et al. 2005) and
467 *Janthinobacterium* sp. J3 isolated from the gut contents of *Batocera horsfieldi* larvae (Zhang
468 et al. 2011).

469 *Sphingobacterium griseoflavum* sp. nov., isolated from the insect *Teleogryllus*
470 *occipitalis* living in deserted crop land (Long et al. 2016) and *Sphingobacterium* isolate
471 exhibiting xylanolytic activity has been isolated from the gut of a cerambycid larva (Zhou et
472 al. 2009). *Alistipes finegoldii* and *Alistipes putredinis* are reported in the gut of medicinal
473 leech (*Hirudo verbena)* (Maltz et al. 2014) and *Alistipes finegoldii* attenuates colitis in mice
474 (Dziarski et al. 2016). *Photorhabdus luminescens* is a bioluminescent entomopathogen which
475 comes under the genus *Photorhabdus*, reported in the study (Schmidt et al. 1989).

476 An et al. (2007) reported the presence of *Terribacillus halophilus* in various insects,
477 even though their role is unknown. *Enterococcus* sp. found in *Bemisia* was reported to
478 produce cyanide oxygenase and utilize cyanide as a nitrogenous growth substance (Fernandez
479 and Kunz 2005). According to Bressan et al. (2008) *SBR proteobacterium* is a pathogen
480 associated with the disease syndrome “basses richesses” of sugar beet in France and are

spread by planthoppers - *Cixius wagneri*, *Hyalesthes obsoletus*, and *Pentastiridius leporinus*.

Concluding remarks

Our study revealed the composition and diversity of bacterial community associated with *B. tabaci* based on Illumina next-generation sequencing of 16S rDNA amplicons. The study was not extrapolated to know the correlation of endosymbiont bacterium and genetic variability in whitefly, as the study conducted by Singh et al. (2012) already ruled out any such possibility. Mining the diversity of bacterial community present in the insects has revealed their role in making *B. tabaci* a successful vector and polyphagous pest of global importance. Our analysis had shown that the specific endosymbiont *Asenophonus* is present only in the heavily cassava mosaic disease infested areas. Insecticidal toxin producing opportunistic bacteria such as *Bacillus thuringiensis* and *Bacillus cereus* were also found in *B. tabaci*. Further studies from more regions and detailed analyses are required to determine the trend in endosymbiont variations based on genetic as well as agro-ecological zone variations. Functional roles of endosymbionts in making *B. tabaci* as a successful vector and invasive plant pest have to be thoroughly studied. Elaborated understanding on endosymbionts could very well be utilized not only for planning alternative pest management strategies but also for enhancing efficiency of beneficial insects.

Acknowledgements

Senior author acknowledges ICAR-CTCRI for granting the permission to carry out the research work. The facilities provided by various departments of College of Horticulture (Kerala Agricultural University), *viz*., Department of Agricultural Entomology, Centre for Plant Biotechnology and Molecular Biology, Department of Agricultural Microbiology and SciGenom, Cochin are gratefully acknowledged.

References

Aksoy, H.M., and Ozman-Sullivan, S.K. 2008. Isolation of *Bacillus megaterium* from *Aphis pomi* (Homoptera: Aphididae) and assessment of its pathogenicity. J. Plant Pathol. **90**(3): 449–452. doi:10.1002/jobm.201500533. PMID:26755240.

Aksoy, S., Chen, X., and Hypsa, V. 1997. Phylogeny and potential transmission routes of midgut-associated endosymbionts of tsetse (*Diptera: Glossinidae*). Insect Mol. Biol. **6**: 183–190. doi:10.1111/j.1365-2583.1997.tb00086.x. PMID:9099582.

Amplicon, P.C.R., Clean-Up, P.C.R., and Index, P.C.R. 2013. 16s metagenomic sequencing library preparation. Available from https://www.illumina.com/content/ dam/illumina-support/documents/documentation/chemistry_documentation/16s/ 16s-metagenomic-library-prep-guide-15044223-b.pdf [accessed 04 February 2019].

Amund, O.O., and Ogunsina, O.A. 1987. Extracellular amylase production by cassava-fermenting bacteria. J. Ind. Microbiol. Biotechnol. **2**(2): 123–127.

An, S.Y., Asahara, M., Goto, K., Kasai, H., and Yokota, A. 2007. *Terribacillus saccharophilus* gen. nov., sp. nov. and *Terribacillus halophilus* sp. nov., spore-forming bacteria isolated from field soil in Japan. Int. J. Syst. Evol. Microbiol. **57**(1): 51–55. doi:10.1099/ijs.0.64340-0. PMID:17220440.

Ateyyat, M.A., Shatnawi, M., and Al-Mazra'awi, M. 2010. Isolation and identification of culturable forms of bacteria from the sweet potato whitefly *Bemesia tabaci* Genn. (Homoptera: Aleyrodidae) in Jordan. Turk. J. Agric. For. **34**(3): 225–234. doi:10.3906/tar-0902-35.

Bartram, A.K., Lynch, M.D., Stearns, J.C., Moreno-Hagelsieb, G., and Neufeld, J.D. 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. Appl. Environ. Microbiol. **77**(11): 3846–3852. doi:10.1128/AEM.02772-10. PMID:21460107.

529 Baumann, P., Moran, N.A., and Baumann, L. 2006. Bacteriocyte-associated endosymbionts
530 of insects. *In* The Prokaryotes: Volume 1: Symbiotic associations, Biotechnology,
531 Applied Microbiology. Edited by Rosenberg E., DeLong E.F., Lory S., Stackebrandt
532 E., and Thompson F. Springer, Berlin, Heidelberg pp.403–438. doi:10.1007/978-3-
533 642-30194-0_19.
534 Bennett, G.M., McCutcheon, J.P., MacDonald, B.R., Romanovicz, D., and Moran, N.A.
535 2014. Differential genome evolution between companion symbionts in an insect-
536 bacterial symbiosis. mBio **5**: e01697. doi:10.1128/mBio.01697-14. PMID:25271287.
537 Bignell, D.E. 2010. Morphology, physiology, biochemistry and functional design of the
538 termite gut: an evolutionary wonderland. *In* Biology of termites: a modern synthesis.
539 Edited by Bignell, D.E., Roisin, Y., and Lo, N. Springer, Netherlands. pp. 375–412.
540 doi:10.1007 / 978-90-481-3977-4_14.
541 Bressan, A., Sémétey, O., Nusillard, B., Clair, D., and Boudon-Padieu, E. 2008. Insect
542 vectors (Hemiptera: Cixiidae) and pathogens associated with the disease syndrome
543 “basses richesses” of sugar beet in France. Plant Dis. **92**(1): 113–119. doi:10.1094/
544 PDIS-92-1-0113. PMID:30786390.
545 Brown, S.D., Lamed, R., Morag, E., Borovok, I., Shoham, Y., Klingeman, D.M., et al. 2012.
546 Draft genome sequences for *Clostridium thermocellum* wild-type strain YS and
547 derived Cellulose adhesion-defective mutant strain AD2. J. Bacteriol. **194**: 3290–
548 3291. doi:10.1128/JB.00473-12. PMID:22628515.
549 Brune, A. 2014. Symbiotic digestion of lignocellulose in termite guts. Nat. Rev. Microbiol.
550 **12**: 168–180. doi:10.1038/nrmicro3182. PMID:24487819.
551 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et
552 al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat.
553 Methods, **7**: 335–336. doi:10.1038/nmeth.f.303. PMID:20383131.

554 Chandler, J.A., Lang, J.M., Bhatnagar, S., Eisen, J.A., and Kopp, A. 2011. Bacterial
555 communities of diverse *Drosophila* species: ecological context of a host–microbe
556 model system. PLoS Genetics, **7**(9): e1002272. doi:10.1371/journal.pgen.1002272.
557 PMID: 21966276.
558 Chandrasekara, A., and Kumar, J.T. 2016. Roots and tuber crops as functional foods: a
559 review on phytochemical constituents and their potential health benefits. Int. J. Food
560 Sci. 15p. doi:10.1155/2016/3631647. PMID:27127779.
561 Chiel, E., Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Katzir, N., Inbar, M., et al. 2007.
562 Biotype-dependent secondary symbionts communities in sympatric populations of
563 *Bemisia tabaci*. Bull. Entomol. Res. **97**: 407–413. doi:10.1017/S0007485307005159.
564 PMID:17645822.
565 Colman, D.R., Toolson, E.C., and Takacs-Vesbach, C.D. 2012. Do diet and taxonomy
566 influence insect gut bacterial communities? Mol. Ecol. **21**(20): 5124–5137.
567 doi:10.1111/j.1365-294X.2012.05752.x. PMID:22978555.
568 Davidson, E.W., Segura, B.J., Steele, T., and Hendrix, D.L. 1994. Microorganisms influence
569 the composition of honeydew produced by the silverleaf whitefly, *Bemisia*
570 *argentifolii*. J. Insect Physiol. **40**(12): 1069–1076. doi:10.1016/0022-1910(94)90060-
571 4.
572 Davis, T.S., Crippen, T.L., Hofstetter, R.W., and Tomberlin, J.K. 2013. Microbial volatile
573 emissions as insect semiochemicals. J. Chem. Ecol. **39**(7): 840–859.
574 doi:10.1007/s10886-013-0306-z. PMID:23793954.
575 Delalibera Jr, I., Handelsman, J., and Raffa, K.F. 2005. Contrasts in cellulolytic activities of
576 gut microorganisms between the wood borer, *Saperda vestita* (Coleoptera:
577 Cerambycidae), and the bark beetles, *Ips pini* and *Dendroctonus frontalis*
578 (Coleoptera: Curculionidae). Environ. Entomol. **34**: 541–547. doi:10.1603/0046-

225X-34.3.541.

DeSantis, T.Z., Hugenholtz, P., Keller, K., Brodie, E.L., Larsen, N., Piceno, Y.M., et al. 2006a. NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. Nucleic Acid Res. **34**: 394–399. doi:10.1093/nar/gkl244. PMID:16845035.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., and Keller, K. 2006b. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. **72**: 5069–5072. doi:10.1128/AEM.03006-05. PMID:16820507.

Dillon, B., Valenzuela, J., Don, R., Blanckenberg, D., Wigney, D. I., and Malik, R. 2002. Limited diversity among human isolates of *Bartonella henselae*. J. Clin. Microbiol. **40**: 4691–4699. doi:10.1128/JCM.40.12.4691-4699.2002. PMID:12454174.

Dillon, R., and Charnley, K. 2002. Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota. Res. Microbiol. **153**(8): 503–509. doi:10.1016/S0923-2508(02)01361-X. PMID:12437211.

Dillon, R.J., Webster, G., Weightman, A.J., and Keith, C.A. 2010. Diversity of gut microbiota increases with aging and starvation in the desert locust. Antonie Van Leeuwenhoek, **97**: 69–77. doi:10.1007/s10482-009-9389-5. PMID:19876756.

Dinsdale, A., Cook, L., Riginos, C., Buckley, Y.M., and Barro, P.D. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Ann. Entomol. Soc. Am. **103**(2): 196–208. doi:10.1603/AN09061.

Douglas, A.E. 2015. Multiorganismal insects: diversity and function of resident microorganisms. Annu. Rev. Entomol. **60**: 17–34. doi:10.1146/annurev-ento-010814-020822. PMID:25341109.

Drancourt, M., Bollet, C., Carlioz, A., Martelin, R., Gayral, J.P., and Raoult, D. 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J. Clin. Microbiol. **38**: 3623–3630. PMID:11015374.

Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstädter, J., et al. 2008. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. BMC Biol. **6**: 27. doi:10.1186/1741-7007-6-27. PMID:18577218.

Dziarski, R., Park, S.Y., Kashyap, D.R., Dowd, S.E., and Gupta, D. 2016. Pglyrp-regulated gut microflora *Prevotella falsenii*, *Parabacteroides distasonis* and *Bacteroides eggerthii* enhance and *Alistipes finegoldii* attenuates colitis in mice. PloS One, **11**(1): e0146162. doi:10.1371/journal.pone.0146162. PMID:26727498.

Eichorst, S.A., Kuske, C.R., and Schmidt, T.M. 2011. Influence of plant polymers on the distribution and cultivation of bacteria in the phylum *Acidobacteria.* Appl. Environ. Microbiol. **77**: 586–596. doi:10.1128/AEM.01080-10. PMID:21097594.

El-Assal, S.E.D., Youssef, N.A., and Amin, G.A. 2013. Isolation and identification of locally isolated bacterial strains effective against whitefly *Bemisia tabaci*. Arch. Agron. Soil Sci. **59**(6): 779–790. doi:10.1080/03650340.2012.682221.

Engel, P., and Moran, N.A. 2013. The gut microbiota of insects - diversity in structure and function. FEMS Microbiol. Rev. **37**(5): 699–735. doi:10.1111/1574-6976.12025. PMID:23692388.

Fernandez, R.F., and Kunz, D.A. 2005. Bacterial Cyanide Oxygenises: Is a suite of enzymes catalyzing the scavenging and adventitious utilization of cyanide as a nitrogenous growth substrate. J. Bacteriol. **187**: 6396–6402. doi:10.1128/JB.187.18.6396-6402.2005. PMID:16159773.

627 Geetha, I., Aruna, R., and Manonmani, A.M. 2014. Mosquitocidal *Bacillus*
628 *amyloliquefaciens*: Dynamics of growth and production of novel pupicidal
629 biosurfactant. Indian J. Med. Res. **140**(3): 427–434. PMID:25366212.
630 Gherna, R.L., Werren, J.H., Weisburg, W., Cote, R., Woese, C.R., Mandelco, L., et al. 1991.
631 Notes: *Arsenophonus nasoniae* gen. nov., sp. nov., the causative agent of the son-
632 killer trait in the parasitic wasp *Nasonia vitripennis*. Int. J. Syst. Bacteriol. **41**(4):
633 563–565. doi:10.1099/00207713-41-4-563.
634 Gleave, A.P., Taylor, R.K., Morris, B.A., and Greenwood, D.R. 1995. Cloning and
635 sequencing of a gene encoding the 69-kDa extracellular chitinase of
636 *Janthinobacterium lividum*. FEMS Microbiol. Lett. **131**: 279–288.
637 doi:10.1111/j.1574-6968.1995.tb07788.x. PMID:7557339.
638 Gottlieb, Y., Fein, E.Z., Daube, N.M., Kontsedalov, S., and Skaljac, M. 2010. The
639 transmission efficiency of *Tomato yellow leaf curl virus* by the whitefly *Bemisia*
640 *tabaci* is correlated with the presence of a specific symbiotic bacterium species. J.
641 Virol. **84**(18): 9310–9317. doi:10.1128/JVI.00423-10. PMID:20631135.
642 Grünwald, S., Pilhofer, M., and Höll, W. 2010. Microbial associations in gut systems of
643 wood- and bark-inhabiting longhorned beetles [Coleoptera: Cerambycidae]. Syst.
644 Appl. Microbiol. **33**: 25–34. doi:10.1016/j.syapm.2009.10.002. PMID:19962263.
645 Gueguen, G., Vavre, F., Gnankine, O., Peterschmitt, M., Charif, D., Chiel, E., et al. 2010.
646 Endosymbiont metacommunities, mtDNA diversityand the evolution of the *Bemisia*
647 *tabaci* (Hemiptera: Aleyrodidae) species complex. Mol. Ecol. **19**: 4365–4378.
648 doi:10.1111/j.1365-294X.2010.04775.x. PMID:20723069.
649 Haris, P., Arun, C.S., and Ravindran, A.D. 2014. Production and characterization of
650 microbial alkaline thermostable protease (*Bacillus pumilus*) from deep sea marine

651 fishes, from southern Arabian sea, Kerala, India. J. Int. Acad. Res. Multidiscip. **2**(6):
652 54–64.
653 Harish, E.R., Mani, C., Makeshkumar, T., Ranjith, M.T., and Ambavane, A.R. 2016.
654 Morphometric variations in cassava (*Manihot esculenta* Crantz) whitefly, *Bemisia*
655 *tabaci* (Gennadius) (Hemiptera: Aleyrodidae) from different agro-ecological zones of
656 Kerala, India. J. Root Crops, **42**(2): 90–102.
657 Himler, A.G., Adachi-hagimori, T., Bergen, J.E., Kozuch, A., Kelly, S.E., Tabashnik, B.E., et
658 al. 2011. Rapid spread of a bacterial symbiont. Science, **332**(6026): 254–256.
659 doi:10.1126/science.1199410. PMID:21474763.
660 Hosokawa, T., Kikuchi, Y., Shimada, M., and Fukatsu, T. 2007. Obligate symbiont involved
661 in pest status of host insect. Proc. Biol Sci. **274**: 1979–1984.
662 doi:10.1098/rspb.2007.0620. PMID:17567556.
663 Hunter, M. S., Perlman, S. J., and Kelly, S. E. 2003. A bacterial symbiont in the
664 *Bacteroidetes* induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia*
665 *pergandiella*. Proc. R. Soc. Lond. B Biol. Sci. **270**(1529): 2185–2190.
666 doi:10.1098/rspb.2003.2475. PMID:14561283.
667 Indiragandhi, P., Yoon, C., Yang, J.O., Cho, S., Sa, T.M., and Kim, G.H. 2010. Microbial
668 communities in the developmental stages of B and Q biotypes of sweet potato
669 whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). J. Korean Soc. Appl. Biol.
670 Chem. **53**(5): 605–617. doi:10.3839/jksabc.2010.
671 Ikotun, T., and Hahn, S.K. 1994. Screening cassava cultivars for resistance to the cassava
672 anthracnose disease (CAD). Acta Hortic. **380**: 178–183.
673 doi:10.17660/ActaHortic.1994.380.26.

674 Janda, M.L., and Abbott, S.L. 2007. 16S rRNA gene sequencing for bacterial identification in
675 the diagnostic laboratory: pluses, perils, and pitfalls. J. Clin. Microbiol. **45** (9): 2761–
676 2764. doi:10.1128/JCM.01228-07. PMID:17626177.
677 Jonathan, G., Lundgren, R., Michael, L., and Joanne, C.S. 2007. Bacterial communities
678 within digestive tracts of ground beetles (Coleoptera: Carabidae). Ann. Entomol. Soc.
679 Am. **100**: 275–282. doi:10.1603/0013-8746(2007)100[275:BCWDTO]2.0.CO;2.
680 Jones, R.T., Sanchez, L.G., and Fierer, N. 2013. A cross-taxon analysis of insect-associated
681 bacterial diversity. PLoS One, **8**: e61218. doi:10.1371/journal.pone.0061218.
682 PMID:23613815.
683 Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K., and Fukatsu, T. 2012.
684 Symbiont-mediated insecticide resistance. Proc. Natl. Acad. Sci. USA, **109**: 8618–
685 8622. doi:10.1073/pnas.1200231109. PMID:22529384.
686 Köhler, T., Dietrich, C., Scheffrahn, R.H., and Brune, A. 2012. High-resolution analysis of
687 gut environment and bacterial microbiota reveals functional compartmentation of the
688 gut in wood-feeding higher termites (*Nasutitermes* spp.). Appl. Environ. Microbiol.
689 **78**(13): 4691–4701. doi:10.1128/AEM.00683-12. PMID:22544239.
690 Köhler, T., Stingl, U., Meuser, K., and Brune, A. 2008. Novel lineages of *Planctomycetes*
691 densely colonize the alkaline gut of soil-feeding termites (*Cubitermes* spp.). Environ.
692 Microbiol. **10**: 1260–1270. doi:10.1111/j.1462-2920.2007.01540.x.
693 PMID:18279348.
694 Lai, C.Y., Baumann, P., and Moran, N. 1996. The endosymbiont (*Buchnera* sp.) of the aphid
695 *Diuraphis noxia* contains plasmids consisting of trpEG and tandem repeats of *trpEG*
696 pseudogenes. Appl. Environ. Microbiol. **62**(2): 332–339. PMID:8593038.
697 Long, X., Liu, B., Zhang, S., Zhang, Y., Zeng, Z., and Tian, Y. 2016. *Sphingobacterium*
698 *griseoflavum* sp. nov., isolated from the insect *Teleogryllus occipitalis* living in

699 deserted cropland. Int. J. Syst. Evol. Microbiol. **66**(5): 1956–1961.
700 doi:10.1099/ijsem.0.000970. PMID:26873062.
701 Malathi, V.G., Nair, N.G., and Shantha, P. 1985. Cassava mosaic disease (Technical Bulletin
702 Series 5). Central Tuber Crops Research Institute, Trivandrum. pp. 18.
703 Maltz, M.A., Bomar, L., Lapierre, P., Morrison, H.G., McClure, E.A., Sogin, M. L., et al.
704 2014. Metagenomic analysis of the medicinal leech gut microbiota. Front.
705 Microbiol. **5**: 151. doi:10.3389/fmicb.2014.00151. PMID:24860552.
706 Martin, J.H. 1987. An identification guide to common whitefly pest species of the world
707 (Homoptera: Aleyrodidae). Trop. Pest Manage. **33**: 298–322.
708 doi:10.1080/09670878709371174.
709 McDonald, D.L. 2012. Investigation of an invasive ant species: *Nylanderia fulva* colony
710 extraction, management, diet preference, fecundity, and mechanical vector potential.
711 Ph.D. thesis, Texas Agricultural Mechanical University, Texas, USA.
712 McGenity, T.J., Folwell, B.D., McKew, B.A., and Sanni, G.O. 2012. Marine crude-oil
713 biodegradation: a central role for interspecies interactions. Aquat. Biosyst. **8**: 10.
714 doi:10.1186/2046-9063-8-10. PMID:22591596.
715 Mereschkowsky, K. 1910. Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis,
716 einer neuen Lehre von der Entstehung der Organismen. Biol. Centralbl. **30**: 353–367.
717 Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., et al. 2008. The
718 metagenomics RAST server – a public resource for the automatic phylogenetic and
719 functional analysis of metagenomes. BMC Bioinform. **9**: 386. doi:10.1186/1471-
720 2105-9-386. PMID:18803844.
721 Mohankumar, B. 2007. Agroforestry systems and practices of Kerala. *In* Agroforestry
722 systems and practices of India. Edited by Puri, S., and Panwar, P. New India
723 Publishing Agency, New Delhi pp. 459–483.

724 Mohr, K.I., and Tebbe, C.C. 2006. Diversity and phylotype consistency of bacteria in the guts
725 of three bee species (Apoidea) at an oilseed rape field. Environ. Microbiol. **8**: 258–
726 272. doi:10.1111/j.1462-2920.2005.00893.x. PMID:16423014.
727 Montllor, C.B., Maxmen, A., and Purcell, A.H. 2002. Facultative bacterial endosymbionts
728 benefit pea aphids *Acyrthosiphon pisum* under heat stress. Ecol. Entomol. **27**: 189–
729 195. doi:10.1046/j.1365-2311.2002.00393.x.
730 Morin, S., Ghanim, M., Zeidan, M., Czosnek, H., Verbeek, M., and van den Heuvel, J.F.
731 1999. A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia*
732 *tabaci* is implicated in the circulative transmission of *Tomato yellow leaf curl virus*.
733 Virology, **256**(1): 75–84. doi:10.1006/viro.1999.9631. PMID:10087228.
734 Moro, C.V., Tran, F.H., Raharimalala, F.N., Ravelonandro, P., and Mavingui, P. 2013.
735 Diversity of culturable bacteria including *Pantoea* in wild mosquito *Aedes*
736 *albopictus*. BMC Microbiol. **13**(1): 70. doi:10.1186/1471-2180-13-70.
737 PMID:23537168.
738 Mukhopadhyay, J., Braig, H.R., Rowton, E.D., and Ghosh, K. 2012. Naturally occurring
739 culturable aerobic gut flora of adult *Phlebotomus papatasi*, vector of *Leishmania*
740 *major* in the Old World. PloS One, **7**(5): e35748. doi:10.1371/journal.pone.0035748.
741 PMID:22629302.
742 Nirgianaki, A., Banks, G.K., Frohlich, D.R., Veneti, Z., Braig, H.R., Miller, T.A., et al. 2003.
743 *Wolbachia* Infections of the Whitefly *Bemisia tabaci*. Curr. Microbiol. **47**: 93–101.
744 doi:10.1007/s00284-002-3969-1. PMID:14506854.
745 Oesi-Poku, J., Mbogo, C.M., Palmer, W.J., and Jiggins, F.M. 2012. Deep sequencing reveals
746 extensive variation in the gut microbiota of wild mosquitoes from Kenya. Mol. Ecol.
747 **21**: 5138–5150. doi:10.1111/j.1365-294X.2012.05759.x. PMID:22988916.

748 Oliver, K.M., Russell, J.A., Moran, N.A., and Hunter, M.S. 2003. Facultative bacterial
749 symbionts in aphids confer resistance to parasitic wasps. Proc. Natl. Acad. Sci. USA,
750 **100**(4): 1803–1807. doi:10.1073/pnas.0335320100. PMID:12563031.
751 Oyewole, O.B., and Odunfa, S.A. 1992. Extracellular enzyme activities during cassava
752 fermentation for 'fufu'production. World J. Microbiol. Biotechnol. **8**(1): 71–72.
753 doi:10.1007/BF01200690. PMID:24425340.
754 Pasti, M.B., and Belli, M.L., 1985. Cellulolytic activity of actinomycetes isolated from
755 termites (Termitidae) gut. FEMS Microbiol. Lett. **26**: 107–112. doi:10.1111/j.1574-
756 6968.1985.tb01574.x.
757 Patel, J.B. 2001. 16S rRNA gene sequencing for bacterial pathogen identification in the
758 clinical laboratory. Mol. Diagn. **6**: 313–321. doi:10.1054/modi.2001.29158.
759 PMID:11774196.
760 Petti, C.A., Polage, C.R., and Schreckenberger, P. 2005. The role of 16S rRNA gene
761 sequencing in identification of microorganisms misidentified by conventional
762 methods. J. Clin. Microbiol. **43**: 6123–6125. doi:10.1128/JCM.43.12.6123-
763 6125.2005. PMID:16333109.
764 Potrikus, C.J., and Breznak, J.A. 1981. Gut bacteria recycle uric acid nitrogen in termites: a
765 strategy for nutrient conservation. Proc. Natl. Acad. Sci. USA, **78**(7): 4601–4605.
766 doi:10.1073/pnas.78.7.4601. PMID:16593064.
767 Priya, N.G., Ojha, H., Kajla, M.K., Raj, A., and Rajagopal, R. 2012. Host plant induced
768 variation in gut bacteria of *Helicoverpa armigera*. PloS One, **7**(1): e30768.
769 doi:10.1371/journal.pone.0030768. PMID:22292034.
770 Raina, H.S., Rawal, V., Singh, S., Daimei, G., Shakarad, M., and Rajagopal, R. 2015.
771 Elimination of *Arsenophonus* and decrease in the bacterial symbionts diversity by

antibiotic treatment leads to increase in fitness of whitefly, *Bemisia tabaci*. Infect. Genet. Evol. **32**: 224–230. doi:10.1016/j.meegid.2015.03.022. PMID:25801610.

Rana, V.S., Singh, S.T., Priya, N.G., Kumar, J., and Rajagopal, R. 2012. *Arsenophonus GroEL* interacts with *CLCuV* and is localized in midgut and salivary gland of whitefly *B. tabaci*. PLoS One, **7**(8): e42168. doi:10.1371/journal.pone.0042168. PMID:22900008.

Ranjith, M.T., ManiChellappan., Harish, E.R., Girija, D., and Nazeem, P.A. 2016. Bacterial communities associated with the gut of tomato fruit borer, *Helicoverpa* armigera (Hübner) (Lepidoptera: Noctuidae) based on Illumina Next-Generation Sequencing. J. Asia Pac. Entomol. **19**(2): 333–340. doi:10.1016/j.aspen.2016.03.007.

Raymond, B., Johnston, P.R., Nielsen, L.C., Lereclus, D., and Crickmore, N. 2010. *Bacillus thuringiensis*: an impotent pathogen? Trends Microbiol. **18**: 189–194. doi:10.1016/j.tim.2010.02.006. PMID:20338765.

Rojas-Pinzón, P. A. and Dussán, J. 2017. Efficacy of the vegetative cells of *Lysinibacillus sphaericus* for biological control of insecticide-resistant *Aedes aegypti*. Parasit. Vectors, **10**(1): 231. doi:10.1186/s13071-017-2171-z. PMID:28490350.

Rosell, R.C., Blackmer, J.L., Czosnek, H., and Inbar, M. 2010. Mutualistic and dependent relationships with other organisms. *In* Bemisia: Bionomics and management of a global pest. Edited by Stansly, P.A., and Naranjo, S.E. Springer, Netherlands, pp. 161–183. doi:10.1007/978-90-481-2460-2_5.

Rosenblueth, M., Sayavedra, L., Sámano-Sánchez, H., Roth, A., and Martínez-Romero, E. 2012. Evolutionary relationships of flavobacterial and enterobacterial endosymbionts with their scale insect hosts (Hemiptera: Coccoidea). J. Evol. Biol. **25**(11): 2357–2368. doi:10.1111/j.1420-9101.2012.02611.x. PMID:22994649.

796 Sabree, Z.L., and Moran, N.A. 2014. Host-specific assemblages typify gut microbial
797 communities of related insect species. SpringerPlus, **3**(1): 138. doi:10.1186/2193-
798 1801-3-138. PMID:24741474.
799 Santos-Garcia, D., Latorre, A., Moya, A., Gibbs, G., Hartung, V., Dettner, K., et al. 2014.
800 Small but powerful, the primary endosymbiont of moss bugs, *Candidatus Evansia*
801 *muelleri*, holds a reduced genome with large biosynthetic capabilities. Genome Biol.
802 Evol. **6**(7): 1875–1893. doi:10.1093/gbe/evu149. PMID:25115011.
803 Schafer, A., Konrad, R., Kuhnigk, T., Kampfer, P., Hertel, H., and Konig, H. 1996.
804 Hemicellulose-degrading bacteria and yeasts from the termite gut. J. Appl. Microbiol.
805 **80**: 471–478. doi:10.1111/j.1365-2672.1996.tb03245.x. PMID:9072518.
806 Schloss, P.D., and Handelsman, J. 2003. Biotechnological prospects from metagenomics.
807 Curr. Opin. Biotechnol. **14**(3): 303–310. doi:10.1016/S0958-1669(03)00067-3.
808 PMID:12849784.
809 Schmidt, T.M., Kopecky, K., and Nealson, K.H. 1989. Bioluminescence of the insect
810 pathogen *Xenorhabdus luminescens*. Appl. Environ. Microbiol. **55**(10): 2607–2612.
811 PMID:2604399.
812 Schrempf, H. 2001. Recognition and degradation of chitin by *Streptomycetes*. Antonie van
813 Leeuwenhoek, **79**: 285–289. doi:10.1023/A:1012058205158. PMID:11816971.
814 Shannon, C.E., and Weaver, W. 1949. *The mathematical theory of communication*. The
815 University of Illinois Press, Urbana, 117 pp.
816 Sharma, P., Sharma, S., Maurya, R.K., De, T.D., Thomas, T., Lata, S., et al. 2014. Salivary
817 glands harbor more diverse microbial communities than gut in *Anopheles*
818 *culicifacies*. Parasit. Vectors, **7**(1): 235. doi:10.1186/1756-3305-7-235.
819 PMID:24886293.
820 Shi, Z.H., Wang, L.L., and Zhang, H.Y. 2012. Low diversity bacterial community and the

821 trapping activity of metabolites from cultivable bacteria species in the female
822 reproductive system of the Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera:
823 Tephritidae). Int. J. Mol. Sci. **13**(5): 6266–6278. doi:10.3390/ijms13056266.
824 PMID:22754363.
825 Singh, S.T., Priya, N.G., Kumar, J., Rana, V.S., Ellango, R., Joshi, A., et al. 2012. Diversity
826 and phylogenetic analysis of endosymbiotic bacteria from field caught *Bemisia tabaci*
827 from different locations of North India based on 16S rDNA library screening. Infect.
828 Genet. Evol. **12**(2): 411–419. doi:10.1016/j.meegid.2012.01.015. PMID:22293464.
829 Sloan, D.B., and Moran, N.A. 2012. Endosymbiotic bacteria as a source of carotenoids in
830 whiteflies. Biol. Lett. **8**(6): 986–989. doi:10.1098/rsbl.2012.0664. PMID:22977066.
831 Song, F., Peng, Q., Brillard, J., Lereclus, D., and LeRoux, C.N. 2014. An insect gut
832 environment reveals the induction of a new sugar-phosphate sensor system in
833 *Bacillus cereus*. Gut Microbes, **5**: 58–63. doi:10.4161/gmic.27092. PMID:24256737.
834 Su, M.M., Guo, L., Tao, Y.L., Zhang, Y.J., Wan, F.H., and Chu, D. 2016. Effects of host
835 plant factors on the bacterial communities associated with two whitefly sibling
836 species. PLoS One, **11**(3): e0152183. doi:10.1371/journal.pone.0152183.
837 PMID:27008327.
838 Su, Q., Oliver, K.M., Pan, H., Jiao, X., Liu, B., Xie, W., et al. 2013. Facultative symbiont
839 *Hamiltonella* confers benefits to *Bemisia tabaci* (Hemiptera: Aleyrodidae), an
840 invasive agricultural pest worldwide. Environ. Entomol. **42**(6): 1265–1271.
841 doi:10.1603/EN13182. PMID:24280594.
842 Thao, M.L., and Baumann, P. 2004. Evolutionary relationships of primary prokaryotic
843 endosymbionts of whiteflies and their hosts. Appl. Environ. Microbiol. **70**(6): 3401–
844 3406. doi:10.1128/AEM.70.6.3401-3406.2004. PMID:15184137.
845 Thomas, T., Gilbert, J., and Meyer, F. 2012. Metagenomics-a guide from sampling to data

analysis. Microb. Inform. Exp. **2**(1): 3. doi:10.1186/2042-5783-2-3. PMID:22587947.

Walters, F.S., and English, L.H. 1995. Toxicity of *Bacillus thuringiensis* δ-endotoxins toward the potato aphid in an artificial diet bioassay. Entomol. Exp. Appl. **77**(2): 211–216. doi:10.1111/j.1570-7458.1995.tb02003.x.

Watanabe, Y., Shinzato, N., and Fukatsu, T. 2003. Isolation of actinomycetes from termites' guts. Biosci. Biotechnol. Biochem. **67**: 1797–1801. doi:10.1271/bbb.67.1797. PMID:12951516.

Werren, J.H. 2012. Symbionts provide pesticide detoxification. Proc. Natl. Acad. Sci. USA, **109**: 8364–8365. doi:10.1073/pnas.1206194109. PMID:22615369.

Xiang, H., Xie, L., Zhang, J., Long, Y.H., Liu, N., Huang, Y.P., et al. 2012. Intracolonial difference in gut bacterial community between worker and soldier castes of *Coptotermes formosanus*. Insect Sci. **19**: 86–95. doi:10.1111/j.1744-7917.2011.01435.x.

Xiao, X., Yin, X., Lin, J., Sun, L., You, Z., Wang, P., et al. 2005. Chitinase genes in lake sediments of Ardley Island, Antarctica. Appl. Environ. Microbiol. **71**: 7904–7909. doi:10.1128/AEM.71.12.7904-7909.2005. PMID:16332766.

Xie, W., Yang, X., Chen, C., Yang, Z., Guo, L., Wang, D., et al. 2018. The invasive MED/Q *Bemisia tabaci* genome: A tale of gene loss and gene gain. BMC Genomics, **19**(1): 68. doi:10.1186/s12864-018-4448-9. PMID:29357812.

Zhang, R., Yang, P., Huang, H., Yuan, T., Shi, P., Meng, K., et al. 2011. Molecular and biochemical characterization of a new alkaline β-propeller phytase from the insect symbiotic bacterium *Janthinobacterium* sp. TN115. Appl. Microbiol. Biotechnol. **92**(2): 317–325. doi:10.1007/s00253-011-3309-0. PMID:21562981.

Zhou, J., Bruns, M.A., and Tiedje, J.M. 1996. DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. **62**: 316–322. PMID:8593035.

871 Zhou, J., Huang, H., Meng, K., Shi, P., Wang, Y., Luo, H., et al. 2009. Molecular and
872 biochemical characterization of a novel xylanase from the symbiotic
873 *Sphingobacterium* sp. TN19. Appl. Microbiol. Biotechnol. **85**(2): 323–333.
874 doi:10.1007/s00253-009-2081-x. PMID:19554324.

Tables

Table 1. Ten major bacteria in each taxonomic category, recorded from P population (Values in the parenthesis are per cent of particular taxonomic category of the bacteria compared to the total identified, when the pre-processed total reads were taken for downstream analyses)

Sl. No.	Phylum	Class	Order	Family	Genus	Species
1	Proteobacteria (87.57)	Gammaproteobacteria (86.47)	Enterobacteriales (85.00)	Enterobacteriaceae (85.01)	*Bacillus* (35.57)	Secondary endosymbiont of *Bemisia tabaci* [non-specified] (70.38)
2	Firmicutes (9.29)	Bacilli (9.14)	Bacillales (8.70)	Bacillaceae (8.25)	*Arsenophonus* (24.69)	*Arsenophonus* endosymbiont of *Bemisia tabaci* (7.19)
3	Bacteroidetes (2.91)	Flavobacteriia (1.70)	Flavobacteriales (1.71)	Flavobacteriaceae (1.71)	*Vibrio* (5.83)	*Bacillus cereus* (4.07)
4	Chlorobi (0.16)	Betaproteobacteria (0.94)	Vibrionales (1.30)	Vibrionaceae (1.30)	*Riemerella* (4.53)	*Bacillus megaterium* (3.78)
5	Actinobacteria (0.02)	Bacteroidia (0.59)	Burkholderiales (0.69)	Oxalobacteraceae (0.65)	*Lysinibacillus* (4.20)	*Bacillus flexus* (1.70)
6	Planctomycetes (0.01)	Sphingobacteriia (0.59)	Bacteroidales (0.60)	Sphingobacteriaceae (0.58)	*Flavobacterium* (2.87)	*Riemerella anatipestifer* (1.32)
7	Verrucomicrobia (0.007)	Chlorobia (0.16)	Sphingobacteriales (0.59)	Bacteroidaceae (0.39)	*Janthinobacterium* (2.94)	*Vibrio harveyi* (1.30)
8	Spirochaetae (0.005)	Deltaproteobacteria (0.16)	Lactobacillales (0.47)	Enterococcaceae (0.38)	*Sphingobacterium* (2.79)	*Lysinibacillus sphaericus* (1.22)
9	Tenericutes (0.004)	Negativicutes (0.15)	Pseudomonadales (0.38)	Pseudomonadaceae (0.37)	*Bacteroides* (1.90)	*Janthinobacterium sp.* J3 (0.86)
10	Acidobacteria (0.002)	Cytophagia (0.03)	Chlorobiales (0.17)	Staphylococcaceae (0.24)	*Enterococcus* (1.83)	*Bacillus pumilus* (0.69)

880 **Table 2.** Ten major bacteria in each taxonomic category, recorded from H population (Values in the parenthesis are per cent of particular
881 taxonomic category of the bacteria compared to the total identified, when the pre-processed total reads were taken for downstream analyses)

Sl. No.	Phylum	Class	Order	Family	Genus	Species
1	Firmicutes (82.67)	Bacilli (82.65)	Bacillales (82.58)	Bacillaceae (77.42)	*Bacillus* (82.27)	*Bacillus thuringiensis* (72.62)
2	Proteobacteria (13.40)	Gammaproteobacteria (16.28)	Enterobacteriales (8.34)	Enterobacteriaceae (8.34)	*Alcanivorax* (8.19)	*Alcanivorax sp. EPR 6* (7.58)
3	Bacteroidetes (0.84)	Bacteroidia (0.71)	Oceanospirillales (7.62)	Alcanivoracaceae (7.62)	*Staphylococcus* (5.58)	*SBR proteobacterium* (6.97)
4	Actinobacteria (0.07)	Flavobacteriia (0.12)	Bacteroidales (0.71)	Staphylococcaceae (5.19)	*Pantoea* (1.13)	*Staphylococcus pasteuri* (3.31)
5	Chlorobi (0.05)	Deltaproteobacteria (0.09)	Vibrionales (0.23)	Bacteroidaceae (0.41)	*Lysinibacillus* (0.76)	*Bacillus amyloliquefaciens* (1.75)
6	Planctomycetes (0.04)	Actinobacteria (class) (0.05)	Lactobacillales (0.16)	Vibrionaceae (0.23)	*Bacteroides* (0.44)	*Staphylococcus sciuri* (1.63)
7	Verrucomicrobia (0.04)	Betaproteobacteria (0.03)	Flavobacteriales (0.12)	Rikenellaceae (0.20)	*Alistipes* (0.22)	*Bacillus megaterium* (1.10)
8	Spirochaetae (0.02)	Chlorobia (0.03)	Actinomycetales (0.05)	Enterococcaceae (0.09)	*Photorhabdus* (0.21)	*Pantoea dispersa* (1.02)
9	Tenericutes (0.01)	Negativicutes (0.02)	Chlorobiales (0.03)	Prevotellaceae (0.07)	*Terribacillus* (0.16)	*Lysinibacillus sphaericus* (0.70)
10	Acidobacteria (0.01)	Cytophagia (0.01)	Pseudomonadales (0.03)	Paenibacillaceae (0.04)	*Enterococcus* (0.10)	*Bacillus pumilus* (0.34)

Legends to Tables and Figures

Table 1. Ten major bacteria in each taxonomic category, recorded from P population (Values in the parenthesis are per cent of particular taxonomic category of the bacteria compared to the total identified, when the pre-processed total reads were taken for downstream analyses)

Table 2. Ten major bacteria in each taxonomic category, recorded from H population (Values in the parenthesis are per cent of particular taxonomic category of the bacteria compared to the total identified, when the pre-processed total reads were taken for downstream analyses)

Figure 1. A graphical representation of reads and OTU proportion (The blue bar represents percentage of total OTUs in the read-count groups. The red bar represents percentage of total read contributed by the OTUs in the read-count group)

Figure 2. Rarefaction analyses of *Bemisia tabaci* bacterial communities (P-population: blue line, H-population: red line)

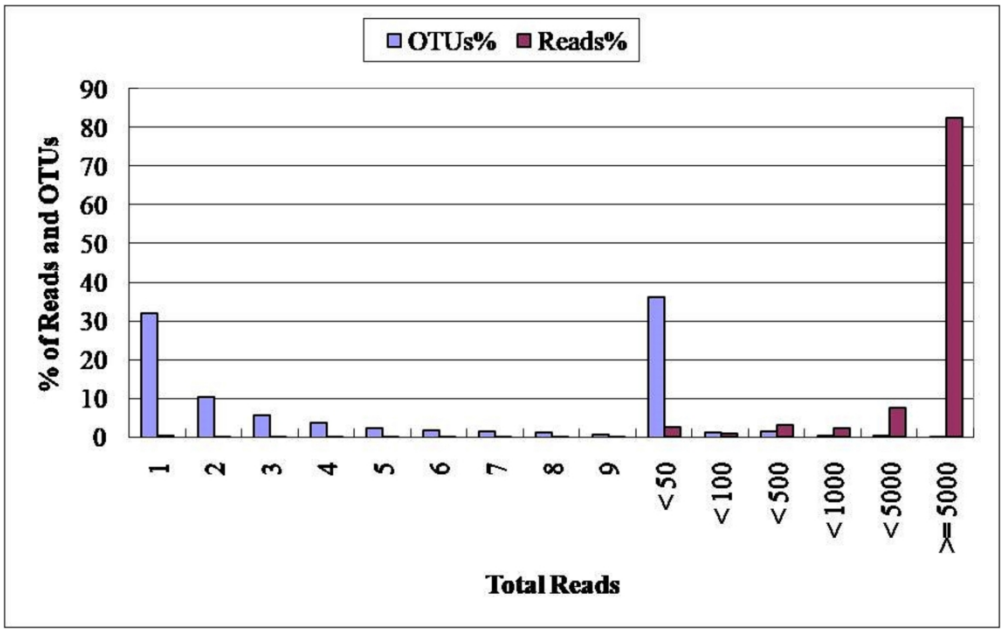
Figure 3. Phylogenetic tree of bacteria at family level constructed in MG-RAST with Illumina sequencing data set (Tree is present with orders (colored slices) and families belong to each orders are given inside colored slices. Magenta box inside the colored slice indicate P-population and green box indicate H-population.The RDP database was used as annotation source, and a minimum identity cutoff (90%) was applied).

Table 1. Ten major bacteria in each taxonomic category, recorded from P population (Values in the parenthesis are per cent of particular taxonomic category of the bacteria compared to the total identified, when the pre-processed total reads were taken for downstream analyses)

Sl. No.	Phylum	Class	Order	Family	Genus	Species
1	Proteobacteria (87.57)	Gammaproteobacteria (86.47)	Enterobacteriales (85.00)	Enterobacteriaceae (85.01)	*Bacillus* (35.57)	Secondary endosymbiont of *Bemisia tabaci* [non-specified] (70.38)
2	Firmicutes (9.29)	Bacilli (9.14)	Bacillales (8.70)	Bacillaceae (8.25)	*Arsenophonus* (24.69)	*Arsenophonus* endosymbiont of *Bemisia tabaci* (7.19)
3	Bacteroidetes (2.91)	Flavobacteriia (1.70)	Flavobacteriales (1.71)	Flavobacteriaceae (1.71)	*Vibrio* (5.83)	*Bacillus cereus* (4.07)
4	Chlorobi (0.16)	Betaproteobacteria (0.94)	Vibrionales (1.30)	Vibrionaceae (1.30)	*Riemerella* (4.53)	*Bacillus megaterium* (3.78)
5	Actinobacteria (0.02)	Bacteroidia (0.59)	Burkholderiales (0.69)	Oxalobacteraceae (0.65)	*Lysinibacillus* (4.20)	*Bacillus flexus* (1.70)
6	Planctomycetes (0.01)	Sphingobacteriia (0.59)	Bacteroidales (0.60)	Sphingobacteriaceae (0.58)	*Flavobacterium* (2.87)	*Riemerella anatipestifer* (1.32)
7	Verrucomicrobia (0.007)	Chlorobia (0.16)	Sphingobacteriales (0.59)	Bacteroidaceae (0.39)	*Janthinobacterium* (2.94)	*Vibrio harveyi* (1.30)
8	Spirochaetae (0.005)	Deltaproteobacteria (0.16)	Lactobacillales (0.47)	Enterococcaceae (0.38)	*Sphingobacterium* (2.79)	*Lysinibacillus sphaericus* (1.22)
9	Tenericutes (0.004)	Negativicutes (0.15)	Pseudomonadales (0.38)	Pseudomonadaceae (0.37)	*Bacteroides* (1.90)	*Janthinobacterium sp.* J3 (0.86)
10	Acidobacteria (0.002)	Cytophagia (0.03)	Chlorobiales (0.17)	Staphylococcaceae (0.24)	*Enterococcus* (1.83)	*Bacillus pumilus* (0.69)

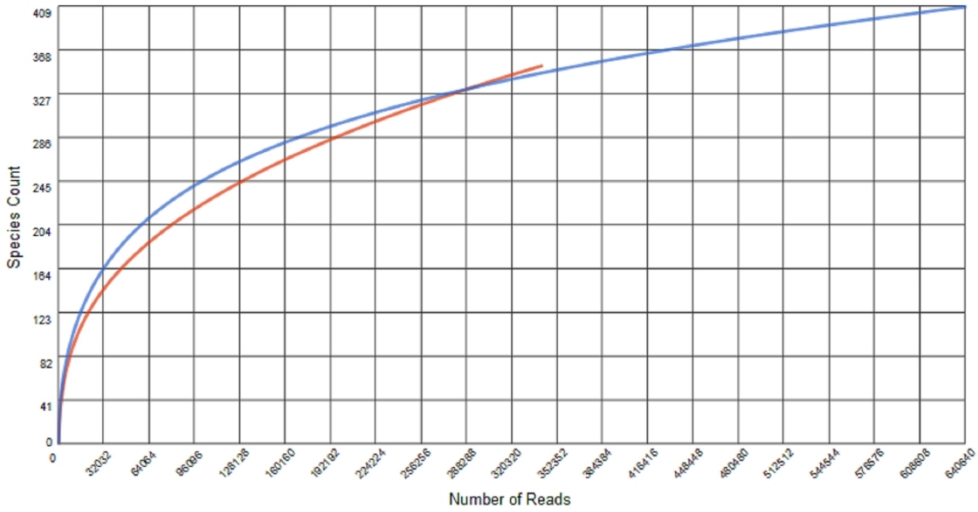
Table 2. Ten major bacteria in each taxonomic category, recorded from H population (Values in the parenthesis are per cent of particular taxonomic category of the bacteria compared to the total identified, when the pre-processed total reads were taken for downstream analyses)

Sl. No.	Phylum	Class	Order	Family	Genus	Species
1	Firmicutes (82.67)	Bacilli (82.65)	Bacillales (82.58)	Bacillaceae (77.42)	*Bacillus* (82.27)	*Bacillus thuringiensis* (72.62)
2	Proteobacteria (13.40)	Gammaproteobacteria (16.28)	Enterobacteriales (8.34)	Enterobacteriaceae (8.34)	*Alcanivorax* (8.19)	*Alcanivorax sp. EPR 6* (7.58)
3	Bacteroidetes (0.84)	Bacteroidia (0.71)	Oceanospirillales (7.62)	Alcanivoracaceae (7.62)	*Staphylococcus* (5.58)	*SBR proteobacterium* (6.97)
4	Actinobacteria (0.07)	Flavobacteriia (0.12)	Bacteroidales (0.71)	Staphylococcaceae (5.19)	*Pantoea* (1.13)	*Staphylococcus pasteuri* (3.31)
5	Chlorobi (0.05)	Deltaproteobacteria (0.09)	Vibrionales (0.23)	Bacteroidaceae (0.41)	*Lysinibacillus* (0.76)	*Bacillus amyloliquefaciens* (1.75)
6	Planctomycetes (0.04)	Actinobacteria (class) (0.05)	Lactobacillales (0.16)	Vibrionaceae (0.23)	*Bacteroides* (0.44)	*Staphylococcus sciuri* (1.63)
7	Verrucomicrobia (0.04)	Betaproteobacteria (0.03)	Flavobacteriales (0.12)	Rikenellaceae (0.20)	*Alistipes* (0.22)	*Bacillus megaterium* (1.10)
8	Spirochaetae (0.02)	Chlorobia (0.03)	Actinomycetales (0.05)	Enterococcaceae (0.09)	*Photorhabdus* (0.21)	*Pantoea dispersa* (1.02)
9	Tenericutes (0.01)	Negativicutes (0.02)	Chlorobiales (0.03)	Prevotellaceae (0.07)	*Terribacillus* (0.16)	*Lysinibacillus sphaericus* (0.70)
10	Acidobacteria (0.01)	Cytophagia (0.01)	Pseudomonadales (0.03)	Paenibacillaceae (0.04)	*Enterococcus* (0.10)	*Bacillus pumilus* (0.34)



A graphical representation of reads and OTU proportion (The blue bar represents percentage of total OTUs in the read-count groups. The red bar represents percentage of total read contributed by the OTUs in the read-count group)

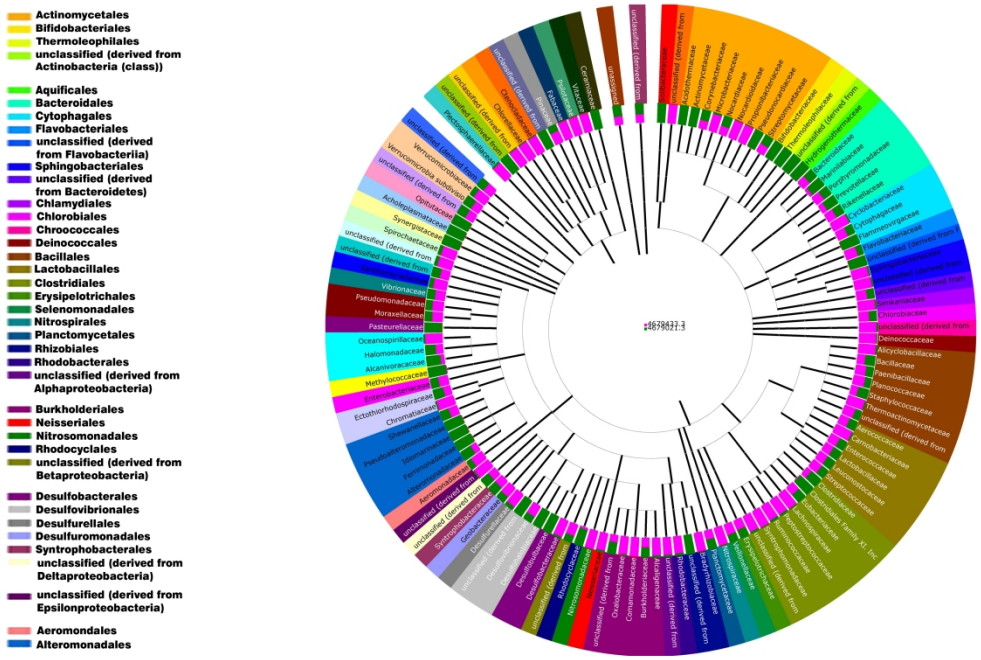
677x423mm (150 x 150 DPI)



rarefaction curve	metagenome	alpha diversity
	4679021.3	3.82
	4679433.3	6.22

Rarefaction analyses of Bemisia tabaci bacterial communities (P-population: blue line, H-population: red line)

952x592mm (96 x 96 DPI)



Phylogenetic tree of bacteria at family level constructed in MG-RAST with Illumina sequencing data set (Tree is present with orders (colored slices) and families belong to each orders are given inside colored slices. Magenta box inside the colored slice indicate P-population and green box indicate H-population.The RDP database was used as annotation source, and a minimum identity cutoff (90%) was applied).

304x203mm (300 x 300 DPI)