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Study on Comparative Diversity of Bacterial Endosymbionts in Invasive Rugose Spiralling Whitefly, *Aleurodicus rugioperculatus* Martin on Different Hosts

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AUTHORS CONTRIBUTION

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ABSTRACT

The contest of the study is to know the influence of host crops on the diversity of bacterial endosymbionts of rugose spiralling whitefly (RSW) *Aleurodicus rugioperculatus* Martin RSW samples were collected from four different hosts (coconut, banana, maize and pongamia) and locations (Bangalore, Doddaballapura, Kolar, Shimoga, Davanagere, Gadag, Tumkur and Mandya). The bacterial symbionts were isolated on nutrient agar media and bacteria were identified by molecular method. RSW found on coconut were recorded highest bacterial species (19) followed by banana (14). The Shannon diversity index and Margleaf richness index bacterial endosymbionts were maximum in coconut (1.73: 0.75) followed by banana (1.47: 0.67). Evenness index of bacterial endosymbionts was maximum in pongamia (0.70) followed by coconut (0.58). Among the bacterial phyla of RSW, Firmicutes were found dominant (69.49%) followed by Proteobacteria (23.72%). Class Bacilli (68.8%) and genus Bacillus (44.26%) were found major in RSW among all the four hosts, followed by g-proteobacteria (16%) and staphylococcus (13.11%). The bacterial endosymbiont diversity of RSW showed a significant difference (Z=2.57) between the four hosts and showed that the host influences the bacterial diversity of RSW.

Keywords : Rugose spiralling whitefly, Endosymbionts, Diversity indices

THE recent invasive insect pest, rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Sternorrhyncha: Aleyrodidae) was described by Martin from Belize in Central America in 2004 based on puparium collected under the leaves of coconut. In India, the RSW was first observed in Pollachi area in Coimbatore district of Tamil Nadu on coconut and first reported in Kottayam from Kerala during July - August 2016 (Sundararaj and Selvaraj, 2017). Presently, the infestation spread over to Karnataka, Kerala and Andhra Pradesh and North India. RSW is morphologically distinguishable from the other whitefly species by its large size (2-3 mm in length) with irregular brown bands on wings and the male possesses a pincer-like structure at the tip of the abdomen. The most preferred hosts are coconut, banana, custard apple, guava and *Canna indica* L. Besides this, interestingly some plant species are preferred for only some life stages of RSW *i.e.,* arecanut, neem, *Parthenium*, mango, tapioca, pepper, ornamental creeper and sapota recorded only the egg stages of the pest (Alagar *et al*., 2020).

The quick adaptation trait of the RSW to changing climate and to the new host is the key factor in their spatiotemporal distribution. The reason behind this adaptation is still unknown. Several research workers worked on the effect of different hosts on the lifecycle of RSW (Pradhan *et al*., 2021) but the works on influence of hosts on its microbial diversity is scarce. The bacterial endosymbionts play an important role in host nutrition, development, fitness, survival, modulation of immune responses and communication

(Devaiah *et al*., 2022) Therefore the present study focusing on microbial diversity of RSW was carried out on four different hosts namely, coconut, banana, maize and pongamia with the hypothesis that host influences the microbial diversity of the RSW.

Material and Methods

Sample Collection

Live RSW adults were collected along with the leaves in a polythene bag with micro holes for aeration, from four different hosts (coconut, banana, maize and pongamia) during 2021-2022 in different locations of Karnataka (Bangalore, Doddaballapura, Kolar, Shimoga, Davanagere, Gadag, Tumkur and Mandya). RSW adults were maintained in the laboratory on the same host for further experimental studies.

Isolation of Gut Bacteria

The collected adults of RSW on different crops and locations were surface sterilized with 70 per cent ethanol for one minute followed by 0.1 per cent sodium hypochlorite for one minute, then rinsed with sterile distilled water 2 to 3 times to remove the external microbes.

The surface sterilized adults were crushed in a microcentrifuge tube using a micro pestle with 1 ml Phosphate Buffer Saline (PBS) solution (pH 7.4). The homogenized samples were centrifuged at 2000 rpm for 10 minutes. Serial dilution of samples was made up of 10-7 dilutions. The aliquot of 1 µl of all the dilutions was plated on Nutrient Agar (NA) for isolating the symbiotic bacteria. An aliquot was spread using a sterilized spreader. The plating was done by the spread plate technique. The plates were incubated at 28 °C for 48 hours. After 24 hours, plates were observed for microbial growth.

DNA Isolation from Gut Bacterial Isolates

Representative colony from colonies showing similar morphology were selected and pure cultured by sub culturing the same media. The pure culture was added to nutrient broth for the multiplication of bacterial cells. Total DNA was extracted from bacterial colonies by inoculating the single colony of bacterial culture in nutrient broth and incubated at 37 °C for 24 hrs. Later, transferred 1.5 ml culture to micro centrifuge tube and centrifuged at 10000 rpm for 3 minutes and collected pellets. Resuspended the pellets on 400 mL sucrose buffer and vertex. Added 32 mL lysozyme, incubated for 10 minutes at 60 °C. Then added 45 mL 10 per cent SDS and 5 mL proteinase, mix well and incubate again in water bath for 10mins at 60 °C. Added 240 mL NaCl and 140 mL freshly prepared 10 per cent CTAB and kept in water bath for 10 minutes. Added 500 mL Chloroform : Isoamyl alcohol (24:1), mixed well and centrifuged at 12000 rpm for 10 minutes. Transfered the upper aqueous phase into new tube and added 50 mL 3M sodium acetate and 300 mL of isopropanol, mixed gently and incubated overnight at -20 °C. Spun at 12000 rpm for 15 min to pellet down the DNA. Added one ml 70 per cent ethanol and spun at 12000 rpm for 10 min (twice). Discarded the supernatant and allowed drying. Resuspended the DNA in 40 mL TE Buffer, added 2 mL RNAse and incubated at 37 °C for 30 min (Swathi *et al*., 2015 and Devaiah *et al*., 2022).

Amplification of 16s rRNA Gene

The 16SrRNA gene was amplified from bacterial colonies by PCR, using universal eubacterial primer pairs eu27. F (5' AGAGTTTGATCCTGGCTCAG-3') and eu1495.R (5'- ACGGCTACCTTGTTA CGACTT3'). PCRs were carried out in 30 mL reactions with each reaction tube containing 0.5 ml of each primer, ~ 15ng of template DNA, 3 mL Taq buffer, 1.5 mL Taq Polymerase. The following condition was used for the PCR reactions: 98 °C for 1 min, 59 °C for 30 seconds and 72 °C for 1 min for 30 cycles and a final extension of 72 °C for 10 min. PCR products were subsequently subjected to Agarose gel electrophoresis. Aliquots (2L) of each PCR product were resolved electrophoretically on one per cent agarose gel using 10X TAE buffer. The PCR products visualized with an UV transilluminator and photographed with a gel documentation system (Gel Doc 200, BIO-RAD, USA) after staining the gel with ethidium bromide (0.5mg mL-1) (Promega), the DNA molecular weight marker, a 1-kbp DNA ladder (Promega) was used to determine the size of the amplified fragments.

16S rRNA Sequencing Analysis

The purified PCR products were sent for sequencing. The nucleotide sequencing of the PCR fragments was performed. The DNA sequences corresponding to 16SrRNA gene, obtained from individual bacteria was reverse complemented using software Bioedit. The obtained sequences were analysed along with the sequences retrieved from the NCBI (National Centre for Biotechnology Information) GenBank using bioinformatics software and bacterial isolates were identified.

Agarose gel electrophoresis was performed and the aliquots of each PCR product was resolved electrophoretically on 1-1.5 per cent agarose gel using 0.5′ TAE buffer. The PCR products were visualized with a UV transilluminator and photographed with a gel documentation system after staining the gel with ethidium bromide (0.5 μg mL^{-1}), the DNA molecular weight marker, a 1-kbp DNA ladder, was used to determine the size of the amplified fragments. The amplicons eluted and sent for sequencing. The obtained sequences were analyzed along with the 16sRNA gene sequences retrieved from the NCBI GenBank and sequences were obtained and accession ID were obtained.

Diversity of Bacterial Endosymbionts

The microbial diversity of RSW on different hosts and between hosts was calculated by using Shanon diversity index (H) and species richness was calculated by using Margalef index of richness (K) species evenness was calculated by using Shanon evenness (E_H) index by using following formulae.

Shanon Diversity Index (*H)* = -Σpi X ln(pi)

Σ : A Greek symbol that means ; 'sum'

ln : Natural log

pi : The proportion of the entire community made up of species i

The higher the value of *H*, the higher the diversity of species in a particular community. The lower the value of *H*, the lower the diversity. A value of *H* = 0 indicates a community that only has one species

Evenness Index (EH) = H / ln (S)

H : The Shannon Diversity Index

S : The total number of unique species

Margalef Index of Richness (K)

K = log S/log N

S indicates the number of species and N indicates the total number of individuals in the sample

The significance test for diversity of endosymbionts of RSW in different hosts was computed by subjecting the data to Wilcoxon Signed Ranks Test for by using the software SPSS 16.0.

Results and Discussion

Samples of RSW collected on different crops from different locations revealed the diversity of gut bacteria varied with hosts and locations (Table 1). Among the hosts, coconut showed more number of bacterial species (19) followed by banana (14) and the least was found in pongamia with three bacterial symbionts. A significant difference (Z=2.5) in microbial diversity of RSW on all four hosts (Table 2). The results were found similar to the work of Saranya *et al*. (2022), recorded 17 gut bacterial isolates of RSW from coconut and 32 isolates from banana. Similarly, 11 bacterial genera were isolated from sweet potato whitefly, *Bemisia tabaci*, which included *Pseudomonas, Deinococcus, Sphingomonas, Acinetobacter, Staphylococcus, Modestobacter, Micrococcus, Bacillus, Kocuria, Microbacterium, Erwinia, Brevibacterium, Exiguobacterium* and Moraxella (Ateyyat *et al*., 2010; Indiragandhi *et al*., 2010 and Visotto *et al*., 2009).

The majority of the bacterial species belonging to the phylum Firmicutes in all the hosts except the pongamia where Actinobacteria was the dominant phylum (Table 3). Among the three phylums, Firmicutes (69.49%) was found to be dominant followed by Proteobacteria (23.72%) (Fig. 1). Of the different classes, Bacilli (68.8%) was the major class in all the hosts followed by *g*-proteobacteria (16%). Among the different genera, *Bacillus* constitutes 44.26 per cent of the bacterial species followed by *Staphylococcus* (13.11%). Similarly, Saranya

TABLE 1
Diversity of bacterial endosymbionts of RSW in four different hosts

Coconut	Banana	Maize	Pongamia
Bacillus sp.	*Bacillus cereus*	*Bacillus* sp.	*Curtobacterium citreum*
Bacillus cereus	*Staphylococcus saprophyticus*	*Terribacillus* sp.	*Metabacillus indicus*
Kocuria palustris	*Bacillus pumilus*	*Priestia aryabhattai*	*Brachybacterium* sp.
Micrococcus aloeverae	*Bacillus* sp.	*Bacillus* sp.	
Brevundimonas sp.	*Staphylococcus* sp.	*Bacillus aerius*	
Staphylococcus haemolyticus	*Klebsiella variicola*	*Bacillus xiamenensis*	
Serratia nematodiphila	*Staphylococcus* sp.	*Priestia megaterium*	
Bacillus subtilis	*Lactococcus lactis*	*Bacillus pumilus*	
Cytobacillus kochii	*Bacillus* sp.		
Acinetobacter pittii	*Acinetobacter soli*		
Pseudomonas monteilii	*Staphylococcus* sp.		
Pseudomonas plecoglossicida	*Enterobacter hormaechei*		
Bacillus pumilus	*Bacillus* sp.		
Bacillus albus	*Bacillus subtilis*		
Bacillus subtilis			
Bacillus licheniformis			
Bacillus sp.			
Pseudomonas sp.			
Bacillus haynesii			

TABLE 2
Number of isolates from the different hosts

Host	No. of microbes	Z Value
Coconut	19	2.570 **
Banana	14	
Maize	8	
Pongamia	3	

** Significant at 1% level

et al. (2022) observed *Bacillus* (80-100%) was the most abundant bacterial genus in the RSW reared on all tested host plants. The genera Bacillus (30%), Acinetobacter (10%) and Exiguobacterium (10%) were observed in the RSW reared on coconut plants.

Diversity Indices

The Shannon diversity index computed showed that, highest bacterial diversity was observed in coconut (1.73) followed by banana (1.47). Interestingly, the Evenness index for pongamia found high (since, number of species is are less) followed by coconut (0.58). Margalef index of richness (K) for coconut was maximum (0.75) followed by banana (0.67) and least was found in pongamia (0.28) (Table 4). Saranya *et al*. (2022) found maximum bacterial diversity and species richness for the isolates of RSW from coconut (2.20 : 3.64), followed by those in the isolates of RSW from banana (2.20: 3.64) as indicated by the Shannon and Margalef diversity indices.

The present study revealed that, host plants influence the gut microbial diversity in host insects. This observation was supported by the findings of Jones *et al.* (2019), who showed that maize and soybean altered the microbial communities in the fall armyworm, *Spodoptera frugiperda*. The gut bacterial populations of *Henosepilachna vigintioctopunctata*

TABLE 3

Bacterial endosymbionts diversity of RSW in four different hosts

Phylum	Class	Species
Firmicutes	Bacilli (42)	*Bacillus* sp.(27)
		Staphylococcus sp. (8)
		Terribacillus sp.(1)
		Priestia (2)
		Niallia nealsonii
		Brevibacillus brevis
		Cytobacillus kochii
		Lactococcus lactis
Proteobacteria	a- proteobacteria (3)	*Sphingobium yanoikuyae*
		Brevundimonas (2)
	b-proteobacteria (2)	*Chromobacterium haemolyticum*
		Acidovorax sp.
	g-proteobacteria (10)	*Acinetobacter* (4)
		Serratia nematodiphila
		Enterobacter hormaechei
		Klebsiella variicola
		Stenotrophomonas maltophilia
Actinobacteria	Actinomycetia (4)	*Kocuria palustris*
		Micrococcus aloeverae
		Curtobacterium (2)

Note: Number present in parenthesis indicate total number of species

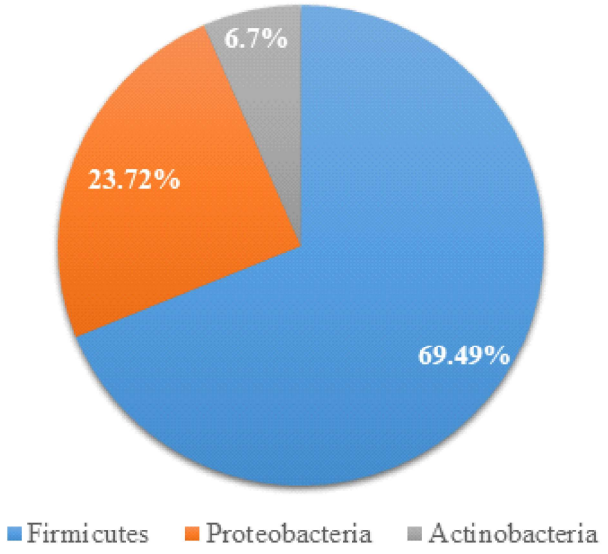

Fig. 1 : Diversity of bacterial endosymbionts at phylum level in RSW on four different hosts

TABLE 4

Diversity indices of bacterial endosymbionts isolated from RSW

Host	H	E_H	K
Coconut	1.73	0.58	0.75
Banana	1.47	0.55	0.67
Maize	0.90	0.43	0.53
Pongamia	0.77	0.70	0.28

H= Shannon diversity index, E_H= Evenness index, K= Margalef index of richness

were influenced by the host plants, *Solanum melongena* (QZ) and *Solanum nigrum* (LK). LK is associated with phylum Cyanobacteria, class Alphaproteobacteria and genus Ochrobactrum, while QZ supports *Bacillus* and *Lactococcus*.

Host plants have a positive impact on the shaping of microbial communities associated with *Spodoptera littoralis* (Tang *et al*. 2012), *Helicoverpa* spp. (Priya *et al*., 2012; Tang *et al*., 2012; Xiang *et al*., 2006), *Lymantria dispar* (Broderick *et al*., 2004; Mason and Raffa 2014) and *Leptinotarsa decemlineata* (Chung *et al*., 2017). Plant characters such as leaf surface, wax composition and the availability of sugars in plants might influence bacterial community composition in the host insect (Lindow and Brandl 2003).

This study enables with deep understanding of the bacterial endosymbionts associated with the RSW on different host crops. This experiment revealed that, RSW collected on the coconut and banana has harboured more diversity of endosymbionts. The change in bacterial diversity in different host crops have made a channel to study about how the endosymbionts are obtained at different crops and eliminated from their body. Understanding the specific functions of each endosymbionts and the transmission patterns will be a suitable area for future research..

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