



# Functional characterization of cultivable gut bacterial communities associated with rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin

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## Abstract

Gut symbiotic bacteria provide protection and nutrition to the host insect. A high reproductive rate and dispersal ability of the rugose spiralling whitefly help this polyphagous species to develop and thrive on many horticultural crops. In this study, we isolated the cultivable gut bacteria associated with rugose spiralling whitefly and demonstrated their role in the host insect. We also studied the influence of antibiotics on the rugose spiralling whitefly oviposition. A total of 70 gut bacteria were isolated from the second nymphal stage of rugose spiralling whitefly reared on coconut, banana, and sapota using seven growth media. From the 70 isolates, chitinase, siderophore (51), protease (44), and Glutathione-S-Transferase producers (16) were recorded. The activities of chitinase, siderophore, protease, and Glutathione-S-Transferase in the gut bacterial isolates of rugose spiralling whitefly ranged from 0.07 to 3.96  $\mu\text{mol}^{-1} \text{min}^{-1} \text{mL}^{-1}$ , 10.01 to 76.93%, 2.10 to 83.40%, and 5.21 to 24.48  $\text{nmol}^{-1} \text{min}^{-1} \text{mL}^{-1} \mu\text{g}^{-1} \text{protein}$ , respectively. The 16S rRNA gene sequence analysis revealed that bacterial genera associated with the gut of rugose spiralling whitefly included *Bacillus*, *Exiguobacterium*, *Acinetobacter*, *Lysinibacillus*, *Arthrobacter*, and *Pseudomonas*. Based on the susceptibility of the gut bacteria to antibiotics, 11 antibiotic treatments were administered to the host plant leaves infested with the nymphal stages. The antibiotics were evaluated for their effect on rugose spiralling whitefly oviposition. Among the antibiotic treatments, carbenicillin (100  $\mu\text{g mL}^{-1}$ ) + ciprofloxacin (5  $\mu\text{g mL}^{-1}$ ) significantly reduced the oviposition (13 eggs spiral<sup>-1</sup>) and egg hatchability (61.54%) of rugose spiralling whitefly. Disruption of chitinase, siderophore, protease, and detoxification enzyme producers and elimination of these symbionts through antibiotics altered the host insect physiology and indirectly affected whitefly oviposition. In conclusion, gut bacteria-based management strategies might be used as insecticides for the effective control of whiteflies.

**Keywords** Antibiotics · Culture-dependent method · Chitinase · Glutathione-S-Transferase · Gut bacteria · Protease · Rugose spiralling whitefly · Siderophore

## Introduction

Rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* was originally described as a pest of coconut in Belize and Mexico in 2004 (Martin 2004). In India, RSW was first found in the coconut farms of the Pollachi area of Tamil Nadu and Palakkad area of Kerala during July–August 2016. RSW infestation was recorded in banana, guava, custard apple, sapota, and other ornamental plants such as areca palm/butterfly palm, oleander, and bird of paradise in India (Selvaraj et al. 2017; Stocks and Hodges 2012). Among these, coconut (40–60%) and banana (25–40%) were severely infested, with 18–43 nymphs/sq.cm present at the midrib region of plants (Selvaraj et al. 2017). Presently, RSW is managed by its introduced natural enemy, *Encarsia*

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*gadeloupae* Viggiani, and systemic neonicotinoid chemical pesticides applied to the leaves, soil, and trunk (Taravati et al. 2018). The application of chemical pesticides might cause the development of resistance and negatively affect the natural enemy. Currently, there is no standard management practice available for controlling RSW. Alternatively, a gut bacteria-based management strategy might be utilized for the sustainable management of whiteflies (Indiragandhi et al. 2007).

Insects contain many types of microorganisms in their intestinal tract, and the interaction between the microorganisms and their hosts varies from symbiosis to pathogenesis (Mrázek et al. 2008). Plant phloem sap is rich in carbohydrates but deficient in nitrogen and essential amino acids (Pandey et al. 2013). The gut bacteria of whiteflies provide nutrients and essential amino acids from food (Douglas 1998). They are also a nitrogen source (Gil et al. 2004) and provide resistance to temperature, insecticides, and natural enemies (Montllor et al. 2002; Oliver et al. 2003; Werren 2012), and immunity (Weiss et al. 2011) to their host. Gut bacteria produce iron-binding compounds called siderophores that are used to obtain iron from the host insect for bacterial growth and development (Ciche et al. 2003). Gut bacterial siderophore protects the host insects from entomopathogens (Indiragandhi et al. 2007). A major constraint in the application of chemical pesticides for pest management is insecticide resistance via detoxification enzymes such as glutathione-S-transferase (Kim et al. 2007; Mohan and Gujar 2003). Insecticides ingested by insects can be detoxified by gut bacterial glutathione-S-transferase, which influences the detoxification process in major insects such as *Helicoverpa armigera* and *Plutella xylostella* (Genta et al. 2006; Indiragandhi et al. 2007; Lauzon et al. 2003; Xiang et al. 2006).

Gut bacteria play a major role in the synthesis of proteins and enzymes responsible for the transmission of viruses in aphids and honeydew secretion in whiteflies. Disruption of these gut bacteria through antibiotics interferes with the transmission of viruses and honeydew secretion by these insects (Davidson et al. 1994; van den Heuvel et al. 1994). RSW can produce large amounts of honeydew, which completely masks plants with sooty mold growth and disrupts photosynthesis (Kumar et al. 2013). Insect midgut, lined with the peritrophic membrane (PM), consists of chitin microfibrils embedded in a protein-carbohydrate matrix (Merzendorfer and Zimoch 2003), which supplies chitin that acts as a source of nitrogen and carbon for gut bacterial growth and benefits the host insect (Indiragandhi et al. 2007). Using the chitinous morphological framework to disrupt the structural part of the insect using enzymes produced by gut bacteria is an eco-friendly method as it is difficult for insects to develop resistance against microbial enzymes. Gut bacterial chitinases are used for the partial degradation of the

old cuticle in insects (Okongo et al. 2019). Culture-dependent isolation of gut bacteria provides information about the functional significance of bacterial isolates and their enzymes can be engineered for pest management (Hernández et al. 2015).

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* are 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* (Lu et al. 2019).

There are no studies on cultivable gut bacterial diversity in rugose spiraling whiteflies and their relation to the host plants since RSW is a recent invasive species. Understanding the effect of antibiotics on the fitness of host insects is a novel strategy for the sustainable management of whitefly through endosymbionts (Costa et al. 1997). This study was designed to reveal the cultivable gut bacterial diversity of rugose spiraling whitefly reared on three host plants along with their functional significance and also to study the effects of antibiotics on RSW oviposition and offspring development.

## Materials and methods

### Mass culturing of rugose spiralling whitefly, *Aleurodicus rugiperculatus*

Coconut leaflets infested with the rugose spiralling whitefly (RSW) were collected from an orchard in Tamil Nadu Agricultural University (TNAU) (11.0123° N, 76.9355° E), Coimbatore, India. The insects were released on mud-potted (pot diameter: 41 cm) plants of coconut, banana, and sapota, which were kept in separate mini net houses (9' × 5' × 7' with nylon net mesh size of 120 µm) and were maintained at an Insectary in the Department of Agricultural Entomology at 31 ± 2 °C, 60–75% RH, and under natural light.

### Isolation of cultivable gut bacteria

The second nymphal stage of RSW damages the host plants considerably. For the isolation of gut bacteria, 50 individuals in the second nymphal stage were collected from coconut, banana, and sapota using a hair-brush under the microscope and starved for 24 h to eliminate the transient gut bacteria. Then, the nymphs were surface sterilized with 70% ethanol followed by 5% sodium hypochlorite for 1 min and washed with sterile water 3–5 times to remove adhering

contaminants. Next, 0.1 mL of the final wash was added to 5 mL of Tryptic soy broth (TSB) and incubated for 48 h at  $28 \pm 2$  °C to ensure surface sterilization. No growth was noticed in TSB. The second nymphal stage of RSW is very small, and it is difficult to dissect the gut. Hence, the whole RSW nymph was taken for gut bacteria isolation after surface disinfection (Malathi et al. 2017). The nymphs were homogenized with 0.1 M phosphate buffer (pH 7.0). The nymphal homogenates were serially diluted in sterile distilled water and 0.1 mL of was spreaded on seven different bacterial growth media, viz., nutrient agar, Luria Bertani, MacConkey agar, tryptic soy agar, endo agar, Reasoner's 2A agar, and MRS agar (M/s. HiMedia Laboratories, Mumbai, India) (de Vries et al. 2001). Petri plates containing gut homogenates were incubated for 48 h at  $28 \pm 2$  °C and monitored for the formation of new colonies at an interval of 24 h. The bacterial colonies were selected based on their colony morphology. Selected colonies were subjected to continuous streaking four-six times to obtain a pure culture, which was confirmed through an examination under a microscope. Purified colonies were stored in 60% glycerol at  $-80$  °C for further experiments (Indiragandhi et al. 2007).

### Molecular characterization

A total of 51 chitinase-producing gut bacteria were grown in nutrient broth for genomic DNA isolation using HipurA<sup>®</sup> Bacterial Genomic DNA Purification Kit (M/s. HiMedia Laboratories, Mumbai, India). Bacterial genomic DNA was then amplified with 16S rRNA gene target universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') by a thermocycler (Mastercycler<sup>®</sup> nexus, Eppendorf India Private Limited). The PCR reaction mixture (25 µL) contained 20 ng of template DNA, 0.3 µL of 1 U *Taq* DNA polymerase (Sigma, India), 2.5 µL of 1 × *Taq* DNA polymerase buffer (Sigma, India), 2 mM magnesium chloride (Sigma, India), 400 µM dNTPs (Sigma, India), 10 pmol of forward and reverse primer, and the final volume was made up with sterilized deionized water. The thermoprofile condition of PCR consisted of initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min and hold at 4 °C. Genomic DNA was viewed and documented using a gel documentation and analysis system (Vilber E-box, Germany).

The fluorescent dye terminator method was used for obtaining the nucleotide gene sequences of 16S rRNA, which was then purified by the Millipore-Montage dye removal kit. Sanger sequencing was performed at the M/s. Barcode Biosciences, Bengaluru, India. The 16S rRNA sequences of the isolates obtained from the automatic sequencer were then aligned and identified using the e-server, EzTaxon ([\[eztaxon-e.ezbiocloud.net/\]\(http://eztaxon-e.ezbiocloud.net/\)\) \(Yoon et al. 2017\) to determine their closest relative. The identified sequences were submitted to NCBI \(<http://www.ncbi.nlm.nih.gov/blast/>\) under the accession numbers MN782273-MN782284, MN784432-MN784435, MN907648-MN907660, MT027239, and MN907663-MN907689 \(Table 2\). Based on the molecular confirmation of the bacterial isolates and the representative isolates of each species, 16 bacterial species were selected for glutathione-S-transferase and antibiotic susceptibility experiments.](http://</a></p>
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### Diversity indices

The Shannon diversity index ( $H'$ ) and the Margalef index of richness ( $K$ ) were calculated using the equations  $H' = -\sum (P_i) \ln(P_i)$  and  $K = \log S / \log N$ , respectively. In the equation to calculate the Margalef index,  $S$  indicates the number of species, and  $N$  indicates the total number of individuals in the sample (Iglesias-Rios and Mazzoni 2014).

### Screening of chitinase-producing gut bacteria

Gut bacterial isolates of RSW obtained from the three host plants were screened based on their chitinolytic activity. The bacterial isolates were inoculated in a nutrient broth supplemented with 0.3% colloidal chitin and incubated at  $28 \pm 2$  °C for 24 h. Colloidal chitin was prepared as described by Rodriguez-Kabana et al. (1983). The crude enzyme solution was collected by centrifugation at 4 °C and  $5500 \times g$  for 20 min. The reaction mixture consisted of 0.3% colloidal chitin (0.1 mL), an enzyme solution (0.1 mL), and 0.1 M McIlvaine buffer of pH 6.0 (0.2 mL). The solution was incubated for 25 min at 35 °C. The reaction was terminated by placing the solution in a water bath for 15 min and adding 2.0 mL of 1.5 mmol potassium ferricyanide. The absorbance was measured using a spectrophotometer (<sup>®</sup>Spectrophotometer 166, Systronics, India) at 420 nm. Known concentrations of N-Acetyl glucosamine (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µmol) were used to generate a standard curve for calculating the enzyme activity. One unit of enzyme activity, i.e., the amount of enzyme required to release 1 µmol of N-Acetyl glucosamine  $\text{min}^{-1}$  (Wiwat et al. 2000), was calculated. The bacterial isolates were screened based on their chitinolytic activity and subjected to further experiments.

### Siderophore production assay

Siderophore-producing bacterial isolates were identified using the ChromAzuroil S (CAS) agar plate method as per Milagres et al. (1999). The autoclaved basal agar medium and CAS indicator solution were allowed to cool to 50 °C. The glucose solution (50%) was prepared and autoclaved. Once cooled, 2 mL of the 50% glucose solution was added

to the autoclaved basal agar medium. Then, 10 mL of the CAS indicator solution was added carefully along the walls of the flask with constant stirring. Once mixed thoroughly, the resulting CAS agar medium (100 mL) was plated in sterile plates. Next, 10  $\mu\text{L}$  of culture ( $1 \times 10^7$  cfu  $\text{mL}^{-1}$ , 24 h old) was spotted on CAS agar plates and incubated for 48 h at  $28 \pm 2$  °C. The production of siderophore was indicated by the appearance of a zone with an orange halo around the spotted colonies. The production of siderophore was calculated by the following formula,

$$\text{Siderophore(\%)} = \frac{\text{Diameter of the orange halo zone}}{\text{Diameter of the colony}} \times 100$$

### Detection of protease activity

The protease enzyme activity of the bacterial isolates was determined using a skim milk agar medium (Cattelan et al. 1999). The medium consisted of peptone (0.1%), NaCl (0.5%), and skim milk (10%). Bacterial culture ( $1 \times 10^7$  cfu  $\text{mL}^{-1}$ , 10  $\mu\text{L}$ , 24 h old) was spotted on skim milk agar plates and incubated for 24 h at  $28 \pm 2$  °C. The diameter of the clearing zone around the spot was measured. The protease activity was calculated by the following formula,

$$\text{Protease(\%)} = \frac{\text{Diameter of the clear zone}}{\text{Diameter of the colony}} \times 100$$

### Estimation of Glutathione-S-Transferase (GST) in gut bacterial isolates of RSW

Gut bacterial isolates of RSW were inoculated in nutrient broth and incubated for 24 h at  $28 \pm 2$  °C. After incubation, the bacterial cells were harvested by centrifugation (8000  $\times g$  for 1 min) and washed twice with buffer K. Then, the cell pellets were suspended in 3–4 volumes of buffer K and subjected to sonication for cell disruption in three 20-s bursts at 50% power and 0 °C. The disrupted cell homogenates were centrifuged for 5 min at 14,000  $\times g$  and 10 °C. Bacterial GST was estimated through the CDNB (1-chloro-2, 4-dinitrobenzene) substrate method as per Lau et al. (1980) and Zablutowicz et al. (1995). The reaction volume (2 mL) contained 1.0 mmol of CDNB and 1.0 mmol of reduced glutathione. The reaction mixture was vortexed and observed spectrophotometrically ( $^{\circ}$ Spectrophotometer 166, Systronics, India) at 340 nm for 5.0 min. This experiment was replicated thrice and control reactions (without enzyme) were maintained conjugation. A unit of enzyme activity ( $\mu\text{mol}$  of CDNB/DCNB conjugated  $\text{min}^{-1}$   $\text{mg}$  of protein  $^{-1}$ ) was calculated using the extinction coefficient of 9.6  $\text{mM}^{-1}$   $\text{cm}^{-1}$ . Enzyme activity was correlated with the amount of protein

in each sample, and protein concentration was measured by the standard method of Bradford (1976).

### Antibiotic susceptibility test for gut bacterial isolates of RSW

Gut bacteria associated with RSW were subjected to sensitivity tests by the Kirby-Bauer disk diffusion method against different antibiotics (Erythromycin E<sup>15</sup>, Streptomycin S<sup>10</sup>, Rifampicin RIF<sup>5</sup>, Polymyxin-B PB<sup>300</sup>, Vancomycin VA<sup>30</sup>, Cefotaxime CTX<sup>30</sup>, Doxycycline DO<sup>20</sup>, Trimethoprim TR<sup>5</sup>, Ciprofloxacin CIP<sup>5</sup>, Colistin CL<sup>10</sup>, Ampicillin AMP<sup>10</sup>, Nalidixin NA<sup>30</sup>, Bacitracin B<sup>8</sup>, Tetracycline TE<sup>30</sup>, Carbenicillin CB<sup>100</sup>, Kanamycin K<sup>30</sup>, Spectinomycin SPT<sup>100</sup>, Chloramphenicol C<sup>30</sup>, and Novobiocin NV<sup>30</sup>) (M/s. HiMedia Laboratories, Mumbai, India). Gut bacterial isolates of RSW were inoculated in nutrient broth and incubated for 24 h at  $28 \pm 2$  °C. After incubation, the bacterial isolates were spread on nutrient agar plates and allowed to dry for 5 min and each antibiotic disc (HiMedia, India) was placed on the surface of the agar using sterilized forceps and incubated for 24 h at  $28 \pm 2$  °C. Then, the diameter of the inhibition zone was measured and compared to the diameter of the inhibition zone published by the Clinical Laboratory Standards Institute (CLSI) to interpret the sensitivity of the antibiotics.

### Effect of antibiotics on RSW oviposition

Effect of the antibiotic treatment (T1-Carbenicillin 100  $\mu\text{g mL}^{-1}$ ; T2-Ciprofloxacin 5  $\mu\text{g mL}^{-1}$ ; T3-Erythromycin 15  $\mu\text{g mL}^{-1}$ ; T4-Cefotaxime 30  $\mu\text{g mL}^{-1}$ ; T5-Carbenicillin 100  $\mu\text{g mL}^{-1}$  + Ciprofloxacin 5  $\mu\text{g mL}^{-1}$ ; T6-Carbenicillin 100  $\mu\text{g mL}^{-1}$  + Erythromycin 15  $\mu\text{g mL}^{-1}$ ; T7-Carbenicillin 100  $\mu\text{g mL}^{-1}$  + Cefotaxime 30  $\mu\text{g mL}^{-1}$ ; T8-Ciprofloxacin 5  $\mu\text{g mL}^{-1}$  + Erythromycin 15  $\mu\text{g mL}^{-1}$ ; T9-Ciprofloxacin 5  $\mu\text{g mL}^{-1}$  + Cefotaxime 30  $\mu\text{g mL}^{-1}$ ; T10-Erythromycin 15  $\mu\text{g mL}^{-1}$  + Cefotaxime 30  $\mu\text{g mL}^{-1}$ ; T11-control) on the oviposition of RSW was evaluated through a bioassay as per the protocol of the Insecticide Resistance Action Committee susceptibility test O16 (IRAC 2009). The above-mentioned antibiotics were applied on coconut leaves infested with the RSW nymphal stages. Clip cages were placed on antibiotic-treated leaves with RSW in the second nymphal stage to feed and oviposit. The oviposition parameters of RSW, including the number of eggs laid/spiral and the percentage of eggs that hatched into immature stages were recorded.

### Scanning electron microscopy

Fresh, hydrated progeny of antibiotic-treated and control population of whitefly eggs and nymphal stages were placed on sticky carbon tabs on aluminum stubs and sputter-coated with gold and palladium in a ratio of 1:1. The coated



specimens were visualized at 10 kV using a scanning-transmission electron microscope (Quanta 250, FEI, Netherland) in the SEM mode at magnifications of 100× to 1000× and documented as microphotographs.

## Statistical analysis

Data were analyzed by performing analysis of variance (ANOVA), and the means were compared using generalized linear models (GLMs) with Tukey's HSD test. All the analyses were performed using IBM SPSS Statistics 22 (Spss 2013).

## Results

### Gut bacterial isolation

A total of 70 gut bacterial isolates were obtained from the nymphal stages of rugose spiralling whitefly (RSW) cultured on three different host plants, coconut, banana, and sapota, using seven different growth media. No bacterial growth was observed in the MacConkey, MRS, and Endo agar media. From the culture-dependent isolation, we recorded 17 gut bacterial isolates of RSW from coconut, 32 isolates from banana, and 20 isolates from sapota (Table S1). The highest bacterial diversity and species richness were observed in TSA (2.30: 3.90), followed by those in NA (2.08: 3.36), R2A (1.95: 3.08), and LB (1.61: 2.48), respectively. The maximum bacterial diversity and species richness were observed 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) and sapota (1.61: 2.50), as indicated by the Shannon and Margalef diversity indices (Table 1).

*Bacillus* (80–100%) was noted the most in the RSW reared on all the tested host plants. The genera *Bacillus* (30%), *Acinetobacter* (10%), and *Exiguobacterium* (10%) were observed in the RSW reared on coconut plants. *Bacillus* (81%), *Lysinibacillus* (11%), *Arthrobacter* (4%), and *Pseudomonas* (4%) were recorded in the RSW from banana plants. However, only *Bacillus* (100%) was present in the RSW reared on sapota plants. The NA medium supported the isolation of *Bacillus* (87%) and *Exiguobacterium* (13%), whereas, in the LB medium, *Bacillus* (64%), *Lysinibacillus* (27%), and *Acinetobacter* (9%) were dominant. *Bacillus* (88%), *Arthrobacter* (6%), and *Pseudomonas* (6%) were isolated in TSA; however, in the R2A growth medium, only *Bacillus* species were reported.

### Functional significance of cultivable gut bacteria isolated from rugose spiralling whitefly

Out of the 70 isolates, 51 gut bacteria were found to be positive for both chitinase and siderophore production and 44

**Table 1** Diversity indices of cultivable bacteria isolated from rugose spiralling whitefly, *A. rugioperculatus*

	Shannon diversity index	Margalef diversity index
Host plants		
Coconut	2.20	3.64
Banana	2.20	3.64
Sapota	1.61	2.50
Media		
Nutrient agar	2.08	3.36
Luria Bertani agar	1.61	2.48
Tryptic Soya Agar	2.30	3.90
R2A agar	1.95	3.08
MacConkey agar	0.00	0.00
MRS agar	0.00	0.00
Endo agar	0.00	0.00

isolates were positive for protease activity. The chitinolytic activity was the highest in *Bacillus siamensis* SBRSW9 (3.96  $\mu\text{mol}^{-1} \text{min}^{-1} \text{mL}^{-1}$ ) followed by that in *Bacillus altitudinis* SBRSW2 (3.01  $\mu\text{mol}^{-1} \text{min}^{-1} \text{mL}^{-1}$ ), which were isolated from the RSW of banana plants; the lowest chitinolytic activity was recorded in *Bacillus altitudinis* SSRSW18 (0.07  $\mu\text{mol}^{-1} \text{min}^{-1} \text{mL}^{-1}$ ) from the RSW of sapota plants. The maximum proteolytic activity was recorded in *Bacillus albus* SCRSW11 (76.93%), followed by that in *Bacillus subtilis* subsp. *Stercoris* SBRSW19 (71.43%). The minimum proteolytic activity was noted in *Bacillus altitudinis* SSRSW13 (10.01%) from RSW collected from coconut, banana, and sapota plants, respectively. The maximum siderophore production was recorded in *Bacillus subtilis* subsp. *Stercoris* SBRSW19 (83.40%) from banana and the minimum siderophore production was in *Bacillus zanthoxyli* SCRSW13 (2.10%) from coconut (Table 2).

The Glutathione-S-Transferase activity (GST) for the gut bacterial isolates of RSW ranged from 5.21 to 24.48  $\text{nmol}^{-1} \text{min}^{-1} \text{mL}^{-1} \mu\text{g protein}^{-1}$  (Fig. 1). The maximum activity of GST was recorded in *Bacillus altitudinis* SCRSW14 (23.42  $\text{nmol}^{-1} \text{min}^{-1} \text{mL}^{-1}$  of 42.9  $\mu\text{g protein}^{-1} \text{min}^{-1}$ ), *Lysinibacillus xylanilyticus* SBRSW13 (24.38  $\text{nmol}^{-1} \text{min}^{-1} \text{mL}^{-1}$  of 52.5  $\mu\text{g protein}^{-1} \text{min}^{-1}$ ), and *Pseudomonas stutzeri* SBRSW22 (23.44  $\text{nmol}^{-1} \text{min}^{-1} \text{mL}^{-1}$  of 49.00  $\mu\text{g protein}^{-1} \text{min}^{-1}$ ), while the minimum enzyme activity was observed in *Acinetobacter refrigerantis* SCRSW5 (5.21  $\text{nmol}^{-1} \text{min}^{-1} \text{mL}^{-1}$  of 20.3  $\mu\text{g protein}^{-1} \text{min}^{-1}$ ) ( $F = 486.199$ ,  $P < 0.001$ ).

### Antibiotic susceptibility test

Sixteen gut bacterial isolates of RSW were tested to assess their susceptibility toward 19 antibiotics. The majority of

**Table 2** Molecular identification and functional significance of cultivable bacteria isolation from rugose spiralling whitefly, *A. rugioperculatus* on three different host plants

S. No.	Isolate designation	Closest match	Similarity (%)	Size of the Sequence (bp)	NCBI Accession no	Media	Chitinase ( $\mu\text{mol}/\text{min}/\text{mL}$ )	Protease (%)	Siderophore (%)
1	SCRSW1	<i>Bacillus licheniformis</i> ATCC 14,580 <sup>T</sup>	94.5	1477	MN782273	NA	0.31 $\pm$ 0.13 <sup>ab</sup>	53.44 $\pm$ 1.02 <sup>d</sup>	5.80 $\pm$ 0.46 <sup>b</sup>
2	SCRSW2	<i>Exiguobacterium mexicanum</i> 8N <sup>T</sup>	97.69	1467	MN782274	NA	0.24 $\pm$ 0.12 <sup>ab</sup>	21.04 $\pm$ 2.80 <sup>b</sup>	24.66 $\pm$ 1.47 <sup>d</sup>
3	SCRSW5	<i>Acinetobacter refrigeratoris</i> WB1 <sup>T</sup>	83.61	1538	MN907646	LB	0.76 $\pm$ 0.16 <sup>b</sup>	0.00 $\pm$ 0.001 <sup>a</sup>	31.33 $\pm$ 3.41 <sup>d</sup>
4	SCRSW7	<i>Bacillus maniponensis</i> BL4-6	90.4	1489	MN782275	TSA	1.91 $\pm$ 0.47 <sup>c</sup>	26.35 $\pm$ 2.95 <sup>b</sup>	8.50 $\pm$ 0.33 <sup>bc</sup>
5	SCRSW8	<i>Bacillus velezensis</i> CR-502 <sup>T</sup>	96.65	1480	MN782276	TSA	0.36 $\pm$ 0.16 <sup>ab</sup>	46.24 $\pm$ 2.10 <sup>d</sup>	6.40 $\pm$ 0.44 <sup>bc</sup>
6	SCRSW10	<i>Bacillus zanthoxyli</i> 1433 <sup>T</sup>	95.87	1490	MN782277	TSA	1.95 $\pm$ 0.04 <sup>c</sup>	26.40 $\pm$ 2.24 <sup>b</sup>	2.30 $\pm$ 0.14 <sup>a</sup>
7	SCRSW11	<i>Bacillus albus</i> N35-10-2 <sup>T</sup>	96.7	1473	MN782278	TSA	0.75 $\pm$ 0.20 <sup>b</sup>	76.93 $\pm$ 0.18 <sup>e</sup>	9.60 $\pm$ 0.98 <sup>bc</sup>
8	SCRSW13	<i>Bacillus zanthoxyli</i> 1433 <sup>T</sup>	96.37	1483	MN782279	R2A	0.15 $\pm$ 0.20 <sup>ab</sup>	43.83 $\pm$ 3.49 <sup>cd</sup>	2.10 $\pm$ 0.22 <sup>a</sup>
9	SCRSW14	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	95.95	1477	MN782280	R2A	0.35 $\pm$ 0.06 <sup>ab</sup>	31.99 $\pm$ 3.77 <sup>bc</sup>	10.50 $\pm$ 0.65 <sup>c</sup>
10	SCRSW16	<i>Bacillus aryabhatai</i> B8W22 <sup>T</sup>	99.05	1,515	MN907647	R2A	0.28 $\pm$ 0.07 <sup>ab</sup>	22.72 $\pm$ 1.02 <sup>b</sup>	7.30 $\pm$ 0.55 <sup>bc</sup>
11	SBRWS2	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	96.56	1469	MN782281	NA	3.01 $\pm$ 0.12 <sup>f</sup>	16.74 $\pm$ 2.05 <sup>bcde</sup>	9.90 $\pm$ 0.79 <sup>bc</sup>
12	SBRWS3	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	96.70	1473	MN782282	NA	1.43 $\pm$ 0.59 <sup>abcd</sup>	16.71 $\pm$ 3.39 <sup>bcde</sup>	7.20 $\pm$ 0.43 <sup>abc</sup>
13	SBRWS4	<i>Bacillus xiamensis</i> HYC-10 <sup>T</sup>	96.15	1479	MN782283	NA	0.56 $\pm$ 0.13 <sup>abcd</sup>	16.67 $\pm$ 2.19 <sup>bcde</sup>	5.60 $\pm$ 0.61 <sup>abc</sup>
14	SBRWS5	<i>Bacillus velezensis</i> CR-502 <sup>T</sup>	96.51	977	MN784432	NA	0.72 $\pm$ 0.13 <sup>abcd</sup>	30.26 $\pm$ 5.49 <sup>efg</sup>	7.20 $\pm$ 0.28 <sup>abc</sup>
15	SBRWS6	<i>Bacillus subtilis</i> subsp <i>stercorarius</i> D7XPNI <sup>T</sup>	94.10	1479	MN782284	NA	1.04 $\pm$ 0.11 <sup>abcd</sup>	13.04 $\pm$ 1.54 <sup>bcd</sup>	48.62 $\pm$ 3.35 <sup>g</sup>
16	SBRWS8	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.86	1528	MN907648	LB	1.68 $\pm$ 0.35 <sup>bcdf</sup>	15.12 $\pm$ 2.60 <sup>bcd</sup>	7.80 $\pm$ 0.49 <sup>abc</sup>
17	SBRWS9	<i>Bacillus siamensis</i> KCTC 13613 <sup>T</sup>	99.66	1529	MN907649	LB	3.96 $\pm$ 1.51 <sup>f</sup>	25.21 $\pm$ 4.25 <sup>cdef</sup>	6.20 $\pm$ 0.64 <sup>abc</sup>

**Table 2** (continued)

S. No.	Isolate designation	Closest match	Similarity (%)	Size of the Sequence (bp)	NCBI Accession no	Media	Chitinase ( $\mu\text{mol}/\text{min}/\text{mL}$ )	Protease (%)	Siderophore (%)
18	SBRSW10	<i>Bacillus altitudinis</i> FJAT-21963 <sup>T</sup>	99.93	1526	MN907650	LB	0.91 $\pm$ 0.13 <sup>abcde</sup>	ND	9.30 $\pm$ 0.97 <sup>abc</sup>
19	SBRSW11	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.93	1529	MN907651	LB	2.15 $\pm$ 0.96 <sup>bcdef</sup>	ND	7.10 $\pm$ 0.44 <sup>abc</sup>
20	SBRSW12	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.86	1492	MN907652	LB	2.22 $\pm$ 0.29 <sup>bcdef</sup>	ND	8.50 $\pm$ 0.64 <sup>abc</sup>
21	SBRSW13	<i>Lysinibacillus xylanilyticus</i> SR-86 <sup>T</sup>	99.39	1,517	MN907653	LB	1.14 $\pm$ 0.38 <sup>abcde</sup>	ND	17.90 $\pm$ 1.23 <sup>e</sup>
22	SBRSW14	<i>Lysinibacillus xylanilyticus</i> SR-86 <sup>T</sup>	99.39	1518	MN907654	LB	1.28 $\pm$ 0.31 <sup>abcde</sup>	38.89 $\pm$ 0.04 <sup>fg</sup>	19.40 $\pm$ 0.75 <sup>e</sup>
23	SBRSW15	<i>Lysinibacillus xylanilyticus</i> SR-86 <sup>T</sup>	99.45	1485	MN907655	LB	0.45 $\pm$ 0.21 <sup>abf</sup>	ND	16.80 $\pm$ 1.34
24	SBRSW17	<i>Bacillus siamensis</i> KCTC 13613 <sup>T</sup>	99.39	1484	MN907656	TSA	1.72 $\pm$ 0.16 <sup>bcdef</sup>	11.09 $\pm$ 2.36 <sup>b</sup>	5.90 $\pm$ 0.35 <sup>abc</sup>
25	SBRSW18	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.12	1512	MN907657	TSA	2.40 $\pm$ 0.34 <sup>bcdef</sup>	14.33 $\pm$ 2.66 <sup>bcd</sup>	6.60 $\pm$ 0.72 <sup>abc</sup>
26	SBRSW19	<i>Bacillus subtilis</i> subsp. <i>stercoris</i> D7XPN1 <sup>T</sup>	99.76	1496	MN907658	TSA	2.07 $\pm$ 0.24 <sup>bcdef</sup>	71.43 $\pm$ 0.81 <sup>i</sup>	83.40 $\pm$ 3.24 <sup>h</sup>
27	SBRSW20	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.93	1526	MN907659	TSA	1.47 $\pm$ 0.26 <sup>abcdef</sup>	ND	7.50 $\pm$ 0.52 <sup>abc</sup>
28	SBRSW21	<i>Arthrobacter nitrophenolicus</i> SJCon <sup>T</sup>	99.72	1,503	MN907660	TSA	1.42 $\pm$ 0.37 <sup>abcdef</sup>	58.35 $\pm$ 1.20 <sup>hi</sup>	21.00 $\pm$ 1.32 <sup>e</sup>
29	SBRSW22	<i>Pseudomonas stutzeri</i> ATCC 17588 <sup>T</sup>	99.88	1527	MT027239	TSA	1.22 $\pm$ 0.24 <sup>abcde</sup>	14.28 $\pm$ 0.95 <sup>bcd</sup>	34.00 $\pm$ 3.48 <sup>f</sup>
30	SBRSW23	<i>Bacillus siamensis</i> KCTC 13613 <sup>T</sup>	99.86	1,530	MN907663	R <sub>2</sub> A	2.02 $\pm$ 0.63 <sup>bcdef</sup>	44.57 $\pm$ 5.15 <sup>gh</sup>	7.30 $\pm$ 0.76 <sup>abc</sup>
31	SBRSW24	<i>Bacillus tequilensis</i> KCTC 13,622 <sup>T</sup>	99.86	1509	MN907664	R <sub>2</sub> A	1.85 $\pm$ 0.27 <sup>bcdef</sup>	66.75 $\pm$ 3.78 <sup>i</sup>	10.70 $\pm$ 0.66 <sup>cd</sup>
32	SBRSW25	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	100	1,489	MN907665	R <sub>2</sub> A	1.12 $\pm$ 0.47 <sup>abcde</sup>	21.15 $\pm$ 2.33 <sup>bcd</sup>	5.30 $\pm$ 0.40 <sup>abc</sup>
33	SBRSW26	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	100	1528	MN907666	R <sub>2</sub> A	1.72 $\pm$ 0.16 <sup>bcdef</sup>	26.10 $\pm$ 2.86 <sup>def</sup>	8.20 $\pm$ 0.57 <sup>de</sup>

**Table 2** (continued)

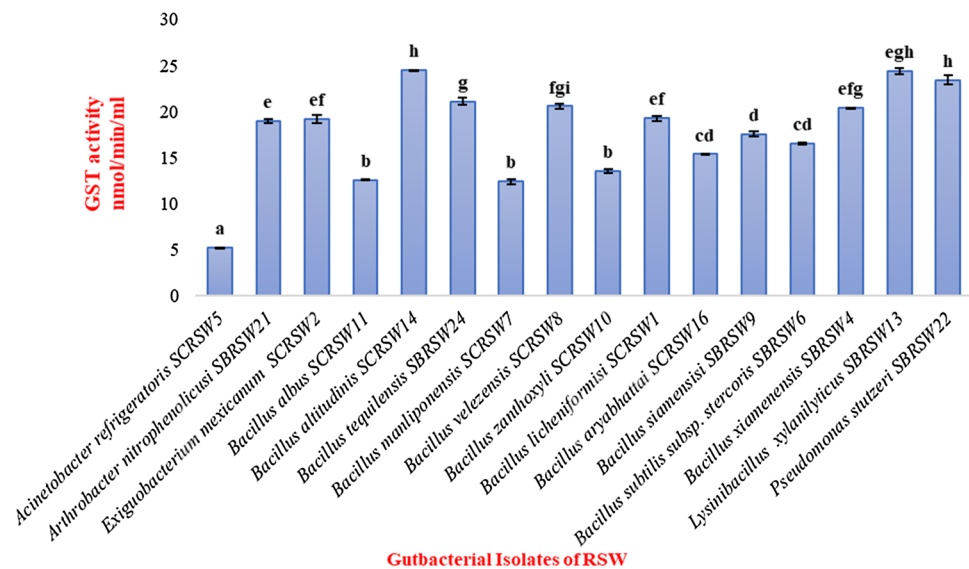
S. No.	Isolate designation	Closest match	Similarity (%)	Size of the Sequence (bp)	NCBI Accession no	Media	Chitinase ( $\mu\text{mol}/\text{min}/\text{mL}$ )	Protease (%)	Siderophore (%)
34	SBRWSW27	<i>Bacillus velezensis</i> CR-502 <sup>T</sup>	98.00	1,466	MN784433	R <sub>2</sub> A	2.82 ± 0.38 <sup>def</sup>	12.48 ± 2.28 <sup>bc</sup>	7.80 ± 0.30 <sup>abc</sup>
35	SBRWSW28	<i>Bacillus siamensis</i> KCTC 13,613 <sup>T</sup>	99.80	1,526	MN907667	R <sub>2</sub> A	2.10 ± 0.47 <sup>bcd</sup>	36.94 ± 4.56 <sup>fg</sup>	6.20 ± 0.49 <sup>abc</sup>
36	SBRWSW29	<i>Bacillus velezensis</i> CR-502 <sup>T</sup>	97.93	1,468	MN784434	R <sub>2</sub> A	2.58 ± 0.31 <sup>cdef</sup>	14.28 ± 2.39 <sup>b</sup>	4.90 ± 0.29 <sup>a</sup>
37	SSRSW1	<i>Bacillus velezensis</i> CR-502 <sup>T</sup>	99.54	922	MN784435	NA	0.40 ± 0.09 <sup>abcd</sup>	20.08 ± 3.58 <sup>abc</sup>	6.20 ± 0.49 <sup>abc</sup>
38	SSRSW4	<i>Bacillus albus</i> N35-10-2 <sup>T</sup>	99.86	1510	MN907668	LB	0.62 ± 0.21 <sup>abcd</sup>	26.67 ± 1.61 <sup>bcd</sup>	7.40 ± 0.44 <sup>abc</sup>
39	SSRSW5	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	100	1509	MN907669	LB	0.27 ± 0.21 <sup>abcd</sup>	15.53 ± 4.60 <sup>ab</sup>	9.20 ± 1.00 <sup>c</sup>
40	SSRSW7	<i>Bacillus albus</i> N35-10-2 <sup>T</sup>	99.93	1489	MN907670	TSA	0.27 ± 0.21 <sup>abcd</sup>	19.05 ± 0.43 <sup>abc</sup>	5.90 ± 1.23 <sup>ab</sup>
41	SSRSW8	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.93	1525	MN907671	TSA	0.05 ± 0.05 <sup>ab</sup>	36.38 ± 2.34 <sup>de</sup>	6.50 ± 0.45 <sup>abc</sup>
42	SSRSW9	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.96	1524	MN907672	TSA	1.13 ± 0.58 <sup>abcd</sup>	27.27 ± 0.76 <sup>bcd</sup>	4.90 ± 0.31 <sup>a</sup>
43	SSRSW10	<i>Bacillus siamensis</i> KCTC 13,613 <sup>T</sup>	99.73	1509	MN907673	TSA	0.55 ± 0.21 <sup>abcd</sup>	42.15 ± 2.43 <sup>e</sup>	7.80 ± 0.80 <sup>bc</sup>
44	SSRSW11	<i>Bacillus albus</i> N35-10-2 <sup>T</sup>	99.66	1512	MN907674	TSA	1.37 ± 0.318 <sup>d</sup>	11.80 ± 2.40 <sup>a</sup>	5.90 ± 0.61 <sup>ab</sup>
45	SSRSW12	<i>Bacillus aryabhatai</i> B8W22 <sup>T</sup>	99.93	1511	MN907675	TSA	1.21 ± 0.47 <sup>bcd</sup>	31.58 ± 3.36 <sup>cde</sup>	7.30 ± 0.45 <sup>abc</sup>
46	SSRSW13	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.86	1518	MN907676	TSA	1.35 ± 0.11 <sup>d</sup>	10.01 ± 2.77 <sup>a</sup>	6.10 ± 0.46 <sup>ab</sup>
47	SSRSW14	<i>Bacillus siamensis</i> KCTC 13,613 <sup>T</sup>	99.86	1509	MN907677	R <sub>2</sub> A	0.61 ± 0.29 <sup>abcd</sup>	25.07 ± 1.59 <sup>bc</sup>	6.50 ± 0.45 <sup>abc</sup>
48	SSRSW15	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.86	1527	MN907678	R <sub>2</sub> A	0.78 ± 0.18 <sup>abcd</sup>	20.01 ± 1.14 <sup>abc</sup>	7.70 ± 0.30 <sup>bc</sup>
49	SSRSW16	<i>Bacillus albus</i> N35-10-2 <sup>T</sup>	99.86	1528	MN907680	R <sub>2</sub> A	1.27 ± 0.36 <sup>cd</sup>	35.09 ± 2.52 <sup>de</sup>	6.90 ± 0.55 <sup>abc</sup>
50	SSRSW17	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.93	1508	MN907681	R <sub>2</sub> A	0.27 ± 0.18 <sup>abcd</sup>	40.01 ± 0.96 <sup>de</sup>	6.20 ± 0.37 <sup>abc</sup>
51	SSRSW18	<i>Bacillus altitudinis</i> 41KF2b <sup>T</sup>	99.80	1486	MN907682	R <sub>2</sub> A	0.07 ± 0.05 <sup>ab</sup>	28.60 ± 1.24 <sup>cde</sup>	8.60 ± 0.94 <sup>bc</sup>

Serial no 1–10 (SCRSW1 – SCRSW 16) indicates RSW bacterial isolates from coconut, Serial no 11–36 (SBRWSW 2– SBRWSW 29) from banana and Serial no 37–51 (SSRSW1–SSRSW18) from sapota. Means in column with the same letter are not significantly different at 0.05 levels (Tukey's HSD test). Values in each column are mean of three replications of  $\pm$  Standard Error (SE)

ND not detected



**Fig. 1** Glutathione-S-Transferase (GST) activity of gut bacterial isolates from rugose spiralling whitefly (RSW). GST enzyme activity was estimated using CDNB substrate. Values in each column are mean of 3 replications of  $\pm$  Standard Error (SE). Means in a column followed by a different letter(s) are significantly different ( $F=486.199$ ,  $P<0.001$ ; Tukey's HSD test)



the gut bacterial isolates were susceptible to most of the tested antibiotics, except Ampicillin AMP<sup>10</sup>, Colistin CL<sup>10</sup>, Kanamycin K<sup>30</sup>, and Polymyxin-B PB<sup>300</sup>. Among the 16 gut bacterial isolates, seven bacterial isolates were susceptible to Erythromycin E<sup>15</sup> and Ciprofloxacin CIP<sup>5</sup>, and six bacterial isolates were susceptible to Carbenicillin CB<sup>100</sup> and Cefotaxime CTX<sup>30</sup>. Hence, Erythromycin E<sup>15</sup>, Ciprofloxacin CIP<sup>5</sup>, Carbenicillin CB<sup>100</sup>, and Cefotaxime CTX<sup>30</sup> were selected based on the maximum zone of inhibition toward bacterial isolates and were used for the antibiotic bioassay (Table S2).

### Antibiotic bioassay

Eleven antibiotic treatments were evaluated for RSW oviposition and egg hatchability (%). The number of eggs laid per spiral (Fig. 3a, b) and the egg hatchability of RSW were significantly reduced by the three antibiotic treatments, viz., CB<sup>100</sup> + CIP<sup>5</sup> (13 eggs spiral<sup>-1</sup> and 61.54%) followed by CIP<sup>5</sup> + CTX<sup>30</sup> (15 eggs spiral<sup>-1</sup> and 80.00%), and CB<sup>100</sup> (16 eggs spiral<sup>-1</sup> and 81.25%) compared to the values of the parameters in the RSW of the control population (29 eggs spiral<sup>-1</sup> and 100%) (Fig. 2).

### Scanning electron microscopy

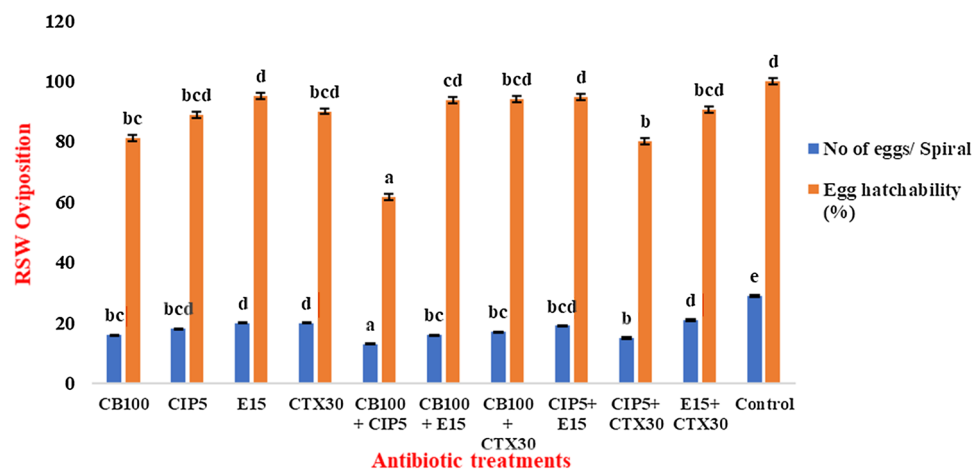
The progeny of the antibiotic-treated RSW showed variation in the morphometry of the egg and nymphal stages. The SEM micrograph showed longer and narrower eggs and nymphs after antibiotic treatment of whiteflies compared to the eggs and nymphs of control whiteflies. The antibiotic-treated eggs and nymphs were longer (320.2  $\mu$ m and 722.9  $\mu$ m) than those in the control population (311.6  $\mu$ m and 675.3  $\mu$ m). Similarly, eggs (121.7  $\mu$ m) and nymphs (431.3  $\mu$ m) were narrower

in the antibiotic-treated samples than the eggs (137.1  $\mu$ m) and nymphs (473.3  $\mu$ m) in the samples of the control population (Fig. 3c–f). Additionally, lesser wax threads were observed on the nymphal progeny of the antibiotic-treated whiteflies than those on the nymphal progeny of the whiteflies in the control population (Fig. 3e, f).

### Discussion

This study revealed the cultivable gut bacteria of rugose spiralling whitefly (RSW) nymphs reared on three different host plants. Based on the molecular confirmation of bacterial isolates, 16 bacterial species were identified, which included *Acinetobacter*, *Arthrobacter*, *Exiguobacterium*, *Bacillus*, *Lysinibacillus*, and *Pseudomonas*. *Bacillus* species were obtained from all selected host plants and isolation media. 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; Visôto et al. 2009).

Varied cultivable gut bacterial populations were present in the nymphal stage of RSW, which might directly or indirectly influence the nutritional role of the host insect. In this study, a high density of *Bacillus* was found in the RSW nymphal stage. *Bacillus* and *Staphylococcus* in the whitefly *Bemisia argentifolii* produce medium-length sugars from derived sucrose and increase the stickiness of honeydew secreted by homopteran insects (Davidson et al. 2000). *Bacillus* species present in the nymphal stage of RSW might be responsible for the production of honeydew,



**Fig.2** Effect of antibiotics on rugose spiralling whitefly oviposition. CB<sup>100</sup>- Carbenicillin 100  $\mu\text{g mL}^{-1}$ , CIP<sup>5</sup>- Ciprofloxacin 5  $\mu\text{g mL}^{-1}$ , CTX<sup>30</sup>- Cefotaxime 30  $\mu\text{g mL}^{-1}$ , E<sup>15</sup>- Erythromycin 15  $\mu\text{g mL}^{-1}$  sprayed on coconut leaves infested with the RSW nymphal stages and clip cages were placed on antibiotic-treated leaves to observe the

effects of antibiotics on RSW oviposition. Values in each column are mean of 3 replications of  $\pm$  Standard Error (SE). Means in a column followed by a different letter(s) are significantly different ( $P=0.05$ ; Tukey's HSD test)

which increases the incidence of sooty mold. RSW produces copious amounts of honeydew, which leads to the growth of the sooty mold fungus, *Capnodium* spp., that disrupts the photosynthesis of the plant, and thus, indirectly affects the quality of coconuts (Mannion 2010; Sundararaj and Selvaraj 2017).

The results suggested that bacterial isolation media and host insect plants influence the cultivable gut bacterial diversity of homopteran insects. The selection of bacterial growth media affects the number and diversity of bacterial phylotypes in the isolation of cultivable bacterial populations from host insects (Davidson et al. 2000). In the present study, various species of cultivable bacteria were retrieved from TSA, NA, R2A, and LB. Cultivable bacterial diversity was the highest in coconut, followed by that in banana and sapota. This was probably because RSW is primarily a pest of coconut.

Similar studies suggested that host plants influence the number and diversity of cultivable gut bacteria in the host insect (Broderick et al. 2004). 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).

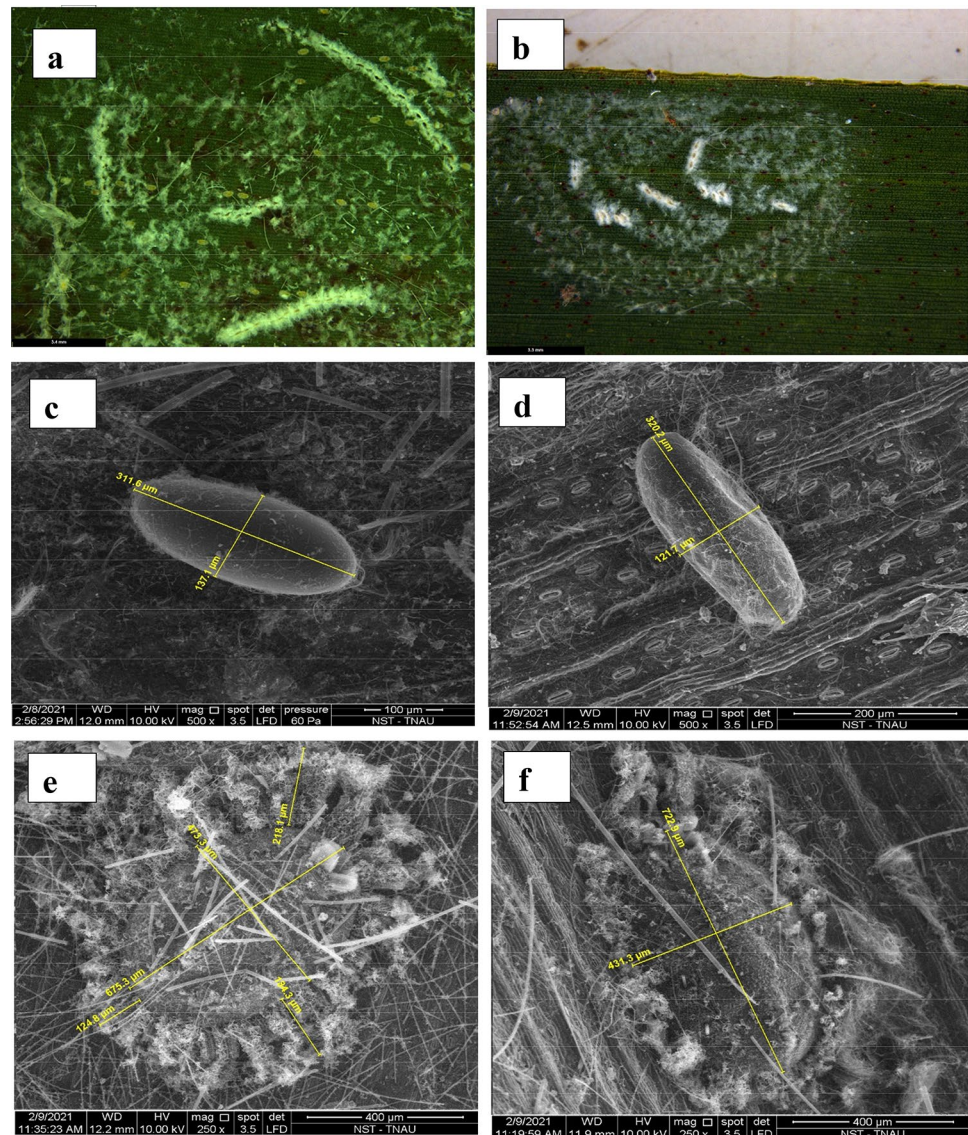
Potential bacterial isolates were screened based on their chitinase-producing nature, and the screened isolates were studied to understand their functional significance. Seventy gut bacterial isolates were found in RSW, of which,

51 isolates produced chitinase and siderophore and 44 isolates exhibited protease activity. Insect midgut lined with the peritrophic membrane (PM) supports digestion and nutrient absorption. Gut bacteria produce chitinase, protease, and siderophores, which provide protection and enhance the growth of host insects (Indiragandhi et al. 2007).

Gut bacteria conserve the thickness of the PM, which affects the diffusion of nutrients across the insect gut (Shen and Jacobs-Lorena 1997). Gut bacteria produce enzymes that can inactivate the immune system of the host insect (Marokházi et al. 2004). In pest management strategy, chitinase and protease-producing gut bacteria were reported to enter the host gut through feeding and disrupt the thickness of the peritrophic membrane; thus, causing a nutrient imbalance in the host insect leading to insect mortality (Krishnamoorthy et al. 2020; Okongo et al. 2019).

Symbiotic bacteria associated with the host insect produce detoxifying enzymes like Glutathione-S-transferase, which provide resistance against insecticides (Baek et al. 2005; Mohan and Gujar 2003). In the present study, glutathione-S-transferase activity in the gut bacterial isolates of RSW ranged from 5.21 to 24.48  $\text{nmol}^{-1} \text{min}^{-1} \text{mL}^{-1} \mu\text{g protein}^{-1}$ . Xenobiotic and endogenous compounds that are detoxified by the glutathione-S-transferase enzyme include DDT, abamectin, and organophosphate insecticides (Hayes and Wolf 1988; Pavlidi et al. 2015). Higher GST activity was reported in *B. tabaci* Q biotype than in B biotype and showed less susceptibility to insecticides (Kim et al. 2007; Seo et al. 2007). Yang et al. (2016) reported a higher GST activity in the thiamethoxam-resistant *B. tabaci* THQR strain compared to the GST activity in the susceptible strain of *B. tabaci* THQS. Similarly, Singh and Walker

**Fig.3** Effect of antibiotics on RSW oviposition and progeny development. (a) image of egg spirals laid by control (b) antibiotic-treated RSW adults; (c) egg laid by control RSW adult (d) egg laid by antibiotic-treated RSW adult; (e) nymphal progeny from control adult (f) nymphal progeny from antibiotic-treated adult. Eggs laid by RSW in antibiotic treatment, carbenicillin  $100 \mu\text{g mL}^{-1}$  + ciprofloxacin  $5 \mu\text{g mL}^{-1}$  treated on coconut leaves showed the least no of eggs per spiral in treated than control viewed in image analyser (Leica M205C) using LAS X software at  $40\times$  magnification (3a and 3b) and egg size, shape changes observed under scanning electron microscope at  $500\times$  magnification (c and d) in antibiotic-treated population. Nymphal progeny of antibiotic-treated RSW adults were observed the wax threads present in the treated population over control (e and f) in scanning electron microscope at  $250\times$  magnification



(2006) reported that *Bacillus* sp. in *Nilaparvata lugens* populations were able to degrade the environmental pollutants, including organophosphorus compounds. Furthermore, Krishnamoorthy et al. (2020) found that *Bacillus* sp. in papaya mealybug might have a role in the detoxification of profenophos and chlorpyrifos OP compounds that were used for the management of the mealybug complex.

The water-soluble antibiotic fumagillin was used for treating *Nosema* infections in *Bombyx occidentalis* (Whittington and Winston 2003) and *Apis mellifera* (Webster 1994), but it showed a negative impact on the developmental stages of *Bombyx occidentalis* and *A. mellifera*. Antibiotic-treated individuals of wood tiger moth, *Parasemia plantaginis*, laid a lesser number of eggs and showed lower immune competence. Antibiotic treatment had deleterious effects on insect populations and reproductive success (Dickel et al. 2016). Similarly, the results of this

study revealed that the number of eggs laid per spiral and egg hatchability were affected in the populations treated with Carbenicillin  $100 \mu\text{g mL}^{-1}$  + Ciprofloxacin  $5 \mu\text{g mL}^{-1}$  relative to the values in the control population (Fig. 3a, b).

Antibiotics with a different mode of action alter the endosymbiont population of the whiteflies (Ahmed et al. 2010; Costa et al. 1997). Carbenicillin disrupted the components required for the synthesis of peptidoglycans in the bacterial cell wall by inactivating the transpeptidase enzyme (Butler et al. 1970). Ciprofloxacin impaired the secretion of DNA gyrase, which is responsible for DNA synthesis (Zweirink and Edison 1986). Costa et al. (1997) reported that carbenicillin disrupted the bacterial cell wall and negatively affected the developmental time and offspring emergence in whiteflies. The treatment of secondary symbionts through antibiotics in *B. tabaci* probably negatively affects the host insect (Ridley et al. 2013; Ruan et al. 2006; Shan et al. 2016).



Rifampicin and oxytetracycline treatment negatively affected the growth and development of the offspring of *B. tabaci* (Costa et al. 1993, 1997; Ruan et al. 2006; Xue et al. 2012).

SEM micrographs showed that the eggs and nymphs of antibiotic-treated whiteflies were longer and narrower, respectively, compared to the eggs and nymphs of the whiteflies in the control population. *Buchnera* elimination with rifampicin, negatively affected the body size, body mass, length, width, fertility, and nutritional requirement of *A. pisum* (Lv et al. 2018). Our results were similar to those of Ruan et al. (2006), who reported that the offspring of *B. tabaci* were longer in the population treated with rifampicin and tetracycline than the offspring of *B. tabaci* in the control population.

Presently, the invasive rugose spiraling whitefly is managed by its natural enemy, the *Encarsia guadeloupae* wasp. The practical utility of this natural enemy in the field was difficult to test due to the lack of knowledge on standard mass-production procedures. Chitinase, siderophore-, and protease-producing gut bacteria and the elimination of symbionts through antibiotics negatively affected the host insect physiology. Rapid and widespread infestation of RSW occurs on host plants due to its high fecundity. Antibiotics negatively affect the development of the progeny of whiteflies. Gut bacteria might be considered as insecticides for the sustainable management of RSW. However, the bio-control efficacy of these isolates is yet to be tested by contact leaf assay or pot culture under field conditions.

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**Author contributions** MS Conducted all the laboratory experiments and manuscript drafting. JSK Conceived the hypothesis designed the experiment and corrected the manuscript. RA Assisted in laboratory experiments and drafted the manuscript.

## Declarations

**Conflict of interest** All authors declare that they have no conflict of interest, no animals or humans are used in this study and all authors accepted the final version submitted to the journal.

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