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Pantothenate mediates the coordination of whitefly and symbiont fitness

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Abstract

Intracellular symbionts in insects often have reduced genomes. Host acquisition of genes from bacteria is an important adaptation that supports symbionts. However, the function of horizontally transferred genes in insect symbiosis remains largely unclear. The primary symbiont *Portiera* housed in bacteriocytes lacks pantothenate synthesis genes: *panB* and *panC*, which is presumably complemented by a fused gene *panB-panC* (hereafter *panBC*) horizontally transferred from bacteria in *Bemisia tabaci* MEAM1. We found *panBC* in many laboratory cultures, and species of *B. tabaci* shares a common evolutionary origin. We demonstrated that complementation with whitefly *panBC* rescued *E. coli* pantothenate gene knockout mutants. *Portiera* elimination decreased the pantothenate level and PanBC abundance in bacteriocytes, and reduced whitefly survival and fecundity. Silencing *PanBC* decreased the *Portiera* titer, reduced the pantothenate level, and decreased whitefly survival and fecundity. Supplementation with pantothenate restored the symbiont titer, PanBC level, and fitness of RNAi whiteflies. These data suggest that pantothenate synthesis requires cooperation and coordination of whitefly PanBC expression and *Portiera*. This host–symbiont co-regulation was mediated by the pantothenate level. Our findings demonstrated that pantothenate production, by the cooperation of a horizontally acquired, fused bacteria gene and *Portiera*, facilitates the coordination of whitefly and symbiont fitness. Thus, this study extends our understanding on the basis of complex host–symbiont interactions.

Introduction

Intracellular symbionts, which include primary and secondary symbionts, are widespread in insects [1, 2]. Primary symbionts can provide specific nutrients that are deficient in the diet and which the host insect cannot synthesize [3–8]. Secondary symbionts may affect insect fitness under certain conditions [2]. Intracellular symbionts, particularly those restricted to specialized host cells (bacteriocytes), have reduced genomes [1].

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Horizontal gene transfer is an important host adaptation that supports and controls insect symbionts [3, 8–10]. Horizontally transferred genes (HTGs) may complement the missing genes involved in synthesis of metabolites by the symbiont [1]. Revealing how genomes from divergent lineages form a functional biosynthetic pathway will help unravel the processes underlying the origin of eukaryotic cells [1, 11].

The important role of HTGs has been increasingly appreciated in insect symbiosis. For example, horizontally transferred *RlpA4* encodes a protein that is transported to the symbiont *Buchnera* [12]; silencing horizontally transferred *amiD* and *ldcA1* decreases the abundance of *Buchnera* in aphids [13]. Horizontally transferred *MurF* encodes a protein that is transported to the symbiont *Moranella* in mealybugs for peptidoglycan synthesis [11]. B vitamins perform various important functions in animals [14]. Animals cannot synthesize these compounds, so they must acquire them from their diet or the microbiome [14]. However, the plant sap diet of Hemiptera is deficient in B vitamins. Horizontally transferred whitefly biotin genes with a bacterial origin can synthesize biotin in the whitefly [15]. Because

functional validation for most HTGs is lacking, the role of HTGs in insect–symbiont interactions and coevolution remains unclear.

Pantothenate is an indispensable B vitamin in animals [14]. Available studies indicate the importance of pantothenate synthesis in insect symbiosis systems [3, 16–19]. The genomes of the symbiont *Buchnera* in the aphid *Acyrthosiphon pisum* [14, 16, 17] and *Moranella* in the mealybug *Planococcus citri* have *panB* and *panC*. However, the genomes of the aphid and mealybug lack these genes [3, 20]. In contrast, the genomes of the primary symbiont *Portiera* in *Bemisia tabaci* MEAM1 and *Carsonella* in the psyllids *Ctenarytaina eucalypti* and *Heteropsylla cubana* lack *panB* and *panC* [18, 19]. These genes are present in the genomes of secondary symbionts of the psyllids *C. eucalypti* and *H. cubana* [19]. Whether the genomes of *C. eucalypti* and *H. cubana* have *panB* and *panC* or not is unknown due to unavailable genome data. But these genes are not present in the genome of the related psyllid species *Pachypsylla venusta* [9]. Fused gene *panB-panC* (hereafter called *panBC*) horizontally transferred from bacteria occurs in *B. tabaci* MEAM1 [18]. These data show the prevalence of *panB* and *panC* in the bacterial symbiosis of sternorrhynchan insects.

The whitefly *B. tabaci* is a complex of more than 35 cryptic species [21–23]. All *B. tabaci* species harbor the primary symbiont "*Candidatus* Portiera aleyrodidarum" (hereafter *Portiera*) in bacteriocytes and may harbor up to four secondary symbiont lineages [24, 25]. Within the *B. tabaci* species complex, *B. tabaci* MEAM1 is a globally important and invasive agricultural pest [26]. In China, *B. tabaci* MEAM1 bears *Portiera* and "*Candidatus* Hamiltonella defensa" (hereafter *Hamiltonella*) in the same bacteriocyte and *Rickettsia* sp. (hereafter *Rickettsia*) in the whole body cavity [15, 27, 28]. The genome of *Portiera* is highly reduced and mainly contains genes involved in synthesis of essential amino acids [29]. *Portiera* also retains genes, except for *panB* and *panC*, involved in pantothenate synthesis. Horizontally transferred *panBC* encoded in the genome of *B. tabaci* MEAM1 is presumably involved in pantothenate synthesis [14, 18]. In contrast, *Hamiltonella* and *Rickettisa* have totally lost the capability for pantothenate synthesis [18], and they have pantothenate symporters that transport pantothenate from the bacteriocytes where the whitefly and *Portiera* cooperate to synthesize this metabolite. However, the manner in which *panBC* contributes to whitefly–symbiont interactions and coevolution is unclear. In this study, the function of the horizontally transferred *panBC* gene in the *B. tabaci* MEAM1 symbiosis system was studied. We reveal that pantothenate provisioned by whitefly *panBC* facilitates the regulation of whitefly symbiosis with *Portiera*.

Materials and methods

Insect rearing and plants

The whitefly *B. tabaci* MEAM1 colony (mtCO1 GenBank accession no. GQ332577) was maintained on cotton plants (*Gossypium hirsutum*, cv. Shiyuan 321) grown in compost supplemented with Miracle-Gro Water Soluble All Purpose Plant Food as described previously [15, 30, 31]. Cotton plants were cultivated to the 6–7 true-leaf stage for use in experiments. For more details, see the Supplementary Text.

Quantitative PCR (qPCR) and RT-PCR

DNA and RNA were extracted following the protocol previously described [15, 32]. Symbionts were quantified by qPCR using the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with 2×SYBR Green Master Mix (Bio-Rad). All primers used in this study are shown in Supplementary Table 1. Relative symbiont density was calculated using the $2^{-\Delta Ct}$ method [33]. RNAs were first strand reverse transcribed using a kit (Bimake, Houston, TX, USA) as described previously [15]. For more details, see the Supplementary Text.

Amino acid sequence alignment and phylogenetic tree analysis

To determine the homologous genes in other *B. tabaci* species, verified sequences of *panBC* of the whitefly *B. tabaci* MEAM1 were subjected to TBLASTX against the genome of *B. tabaci* MED [34] and SSA-ECA (GenBank accession No.: GCA_004919745.1) and transcriptome of *B. tabaci* MEAM [10], MED [35], and Asia II 3 [36]. Amino acid sequence alignments were conducted using BioEdit v7.1.3.0 among the three *B. tabaci* species, *B. aphidicola*, and *E. coli*. To construct the molecular phylogenetic tree, a Bayesian inference (BI) analysis was conducted as described previously [10, 15]. For more details, see the Supplementary Text.

Fluorescence in situ hybridization (FISH)

Localization of *Portiera* in bacteriocytes of female adult whiteflies was investigated by FISH following a previously described protocol [15, 30, 37]. For more details, see the Supplementary Text.

Recombinant enzyme generation and antibody preparation

The recombinant enzyme for whitefly *panBC* was generated, and custom-made polyclonal antibodies against

PanBC (predicted size, 68KD) protein were produced by ProbeGene Life Sciences Co. Ltd. following previously described methods [12, 15, 38]. For more details, see the Supplementary Text.

Immunofluorescence microscopy

Bacteriocytes from *Portiera*-infected and *Portiera*-cured adult female whiteflies as well as dsRNA-injected adult female whiteflies were dissected, fixed, permeabilized, and incubated with antibodies. Images were collected and analyzed on a FV3000 confocal microscope (Olympus, Japan). For more details, see the Supplementary Text.

Pantothenate measurement

A microbiological assay was used for pantothenate quantification in whiteflies using *Lactobacillus plantarum* ATCC 8014 (Beijing Landbridge Technology Limited, Beijing, China) referring to the protocol of [15, 39]. Briefly, 50 female adult whiteflies from each treatment were collected for each of three replicates using a mouth aspirator and flash-frozen in liquid N_2. Insects from each treatment were pooled, weighed, and homogenized in citrate buffer using homogenizer MP FastPrep-24 (MP Biomedicals LLC, Santa Ana, CA, USA) and incubated at 95 °C for 30 min. Then samples were sterilized using a 0.2-μm pore size filter (PALL Life Science, Port Washington, NY, USA), mixed with vitamin B5-deficient pantothenate assay medium (hereafter called as B5DP; Beijing Landbridge Technology Limited, Beijing, China), and inoculated with log-phase *L. plantarum* ATCC 8014. Cultures were incubated for 20 h at 37 °C. Standard concentrations of pantothenate (0–80 ng/mL; Dr. Ehrenstorfer Gmbh, Augsburg, Germany) were mixed with the *L. plantarum* culture to create a standard curve. A negative control consisting of citrate buffer only was subjected to the complete procedure to ensure the retention of the initial pantothenate and a lack of additional pantothenate. An additional negative control included only the assay medium lacking *L. plantarum* to exclude contamination by other bacteria. The growth of *L. plantarum* was measured using a microplate reader (Versa Max Molecular Devices, San Jose, CA, USA) taking absorbance readings at 630 nm. Pantothenate in whiteflies was quantified using the standard curve and normalized to the weight of insects used in the homogenate.

Functional complementation of *E. coli* pantothenate auxotrophs with whitefly *panBC*

To examine the metabolic function of horizontally transferred *panBC*, *E. coli* pantothenate gene (*panB* and *panC*) knockout mutants were generated and functional complementation with whitefly *panBC* was performed. The kanamycin resistance site

was amplified from *E. coli* G11 using knockout primers (Supplementary Table 1). Then pKD46 plasmids expressing Lambda Red recombinase were transformed into the *E. coli* K-12 BW25113 by electroporation. Subsequently, the *E. coli* K-12 BW25113 *panB* and *panC* knockout mutants (i.e., −Δ*panB* and −Δ*panC*) were generated following the Lambda Red protocol as previously described [40–42]. *E. coli* mutants were screened using a LB media agar plate with 0.1-mg/mL kanamycin. PCR amplification was carried out using primers specific for deleted genes, the kanamycin resistance site, and the flanking locus to verify the loss of the parental fragment and gain of the kanamycin resistance site (Supplementary Table 1). The *E. coli* mutants were then made into competent cells by electroporation. The whole CDS region of *panBC* was amplified from *panBC*-PUC57, as described above, and was ligated to the pMD19-T vector. For the functional complement experiments, *E. coli* K-12 mutant cells were transformed with the plasmids pMD19-T-*panBC* and pMD19-T empty vector (negative control), respectively, following previous methods [15, 16]. The *E. coli* wild-type K-12, mutant K-12 and mutant K-12 transformants were grown overnight in LB agar media with 0.1 mg/mL ampicillin at 37 °C. The mutant K-12 transformants were verified by PCR amplification using primers specific for inserted genes. Then *E. coli* wild-type K-12, mutant K-12, and mutant K-12 transformants were grown overnight in pantothenate-deficient B5DP medium (Beijing Landbridge Technology Limited, Beijing, China) at 37 °C. All *E. coli* cells were washed twice in sterile distilled water and resuspended to measure the cell density at OD_{600} using a microplate reader (Versa Max Molecular Devices, San Jose, CA, USA).

Effects of *Portiera* elimination by antibiotic treatment on PanBC level, symbiont localization, pantothenate level, and whitefly performance

Previous studies have demonstrated that treatment with antibiotic rifampicin at 30 μg/ml for 2 days was effective in curing *Portiera*, *Hamiltonella*, and *Rickettsia* in the F1 *B. tabaci* [43, 44]. Thus, to eliminate *Portiera*, hundreds of adult whiteflies of *B. tabaci* (F0, 0–7 days after emergence) were released into each feeding chamber and fed on 25% sucrose solution (w/v) supplemented with the antibiotic rifampicin (BBI Life Sciences, Shanghai, China) dissolved in 5-mM phosphate buffer (pH 7.0), at 30 μg/ml for 2 days as described previously [43, 44]. Control whiteflies were allowed to feed on sucrose solution not supplemented with antibiotics. Following the antibiotic treatment, *B. tabaci* were transferred to cotton plants. F1 female and male adults were collected. The DNA was extracted from eight female adults of *B. tabaci* (at 3–7 days after eclosion) and used for symbiont quantification by qPCR. The F1 *B. tabaci* with reduced *Portiera* titer (−PBt), which were obtained by antibiotic treatment, and control F1 *B. tabaci* (+PBt), which

were obtained by feeding sucrose solution not supplemented with antibiotics, were identified. To test whether *Portiera* elimination affects the PanBC level and symbont localization in bacteriocytes, bacteriocytes of female adults of *B. tabaci* (at 3–7 days after eclosion) were dissected, fixed, permeabilized, and incubated with antibodies and a fluorescent probe for *Portiera* as described above. Three biological replicates were conducted. Images were collected and analyzed on a FV3000 confocal microscope (Olympus, Japan). In parallel, 50 female adult whiteflies (at 3–7 days after eclosion) in each of three biological replicates were collected for pantothenate analysis as described above.

To determine if *Portiera* elimination by antibiotic treatment influences whitefly performance, 20 female adult whiteflies within 3 days post emergence per replicate of +PBt and −PBt lines were transferred onto a cotton leaf disk kept on a 1.5% agar plate at $26 \pm 2\,°C$, with 14:10 h (*L:D*) photoperiod and 60–80% RH. Four biological replicates were conducted. The mortality of injected female whiteflies was recorded at days 3 and 6. In addition, one pair of female and male adult whiteflies at 2 h post emergence from +PBt and −PBt lines were released onto one cotton leaf disk kept on 1.5% agar plate, and allowed to lay eggs for 6 days. Ten biological replicates were conducted. After 6 days, eggs were recorded.

dsRNA preparation

dsRNAs specific to whitefly *panBC* (ds*panBC*) and *GFP* (ds*GFP*) were synthesized using a T7 RiboMAX™ Express RNAi System kit (Promega, USA), following the manufacturer's instructions. For more details, see the Supplementary Text.

Effects of silencing *panBC* on PanBC level, symbiont titer, pantothenate levels, and whitefly performance

To investigate whether silencing of horizontally transferred *panBC* gene influences PanBC level, symbiont titer, and pantothenate levels, ~3000 female adult whiteflies infected with *Portiera* within 4–6 days after emergence were injected with 1.5-µg/µL ds*panBC* in injection buffer using an Eppendorf microinjection system (Hamburg, Germany) referring to a previously described method with some modifications [45]. Control whiteflies were injected with ds*GFP*. The average injection volume used was 10 nl. After injection, whiteflies were transferred onto cotton leaf disks kept on 1.5% agar plates in the incubator at $26 \pm 2\,°C$, with a 14:10 h (*L:D*) photoperiod and 60–80% RH. The survival rate of injected whiteflies was 85–90% at 24 h after injection, showing that the microinjection protocol had little physical effect on the whiteflies. To examine whether silencing whitefly *panBC* affects the PanBC level and

symbiont localization in bacteriocytes, whiteflies were collected at days 1, 3, 5, and 7, and the whitefly bacteriocytes were dissected, fixed, permeabilized, and incubated with antibodies and a fluorescent probe for *Portiera* as described above. Three biological replicates were conducted. Images were analyzed on a FV3000 confocal microscope (Olympus, Japan). To test whether silencing whitefly *panBC* influences symbiont abundance, DNA was extracted from individual female adult whiteflies for each of ten biological replicates at day 3 after whiteflies were microinjected with ds*panBC*. Then, qPCR was performed as described above. In parallel, 50 female adult whiteflies in each of three biological replicates were collected for pantothenate analysis as described above.

To determine if *panBC* silencing influences whitefly performance, ~700 female adult whiteflies infected with *Portiera* within 4 days after emergence were injected using the microinjection procedures described above. After injection, 30 female adults per replicate of *panBC*-injected and ds*GFP*-injected whiteflies were transferred onto a cotton leaf disk kept on a 1.5% agar plate at $26 \pm 2\,°C$, with 14:10 h (*L:D*) photoperiod and 60–80% RH. Five biological replicates were conducted. The mortality of injected female whiteflies was recorded at days 1, 3, 5, and 7. In addition, after injection, individual whiteflies were transferred onto cotton leaf disks as described above. Egg numbers were recorded for living whiteflies with 13 biological replicates of individuals at day 7 post injection.

Effects of pantothenate supplementation on symbiont localization, PanBC level, and whitefly performance

To investigate whether pantothenate supplementation restores the symbiont localization and PanBC level of dsRNA-injected whiteflies, ~500 female adult whiteflies within 4–6 days after emergence were microinjected with ds*panBC* and ds*GFP*. After recovery on a cotton leaf disk for 12 h, these whiteflies were fed 30% (w/v) sucrose solution supplemented with or without pantothenate at a final concentration of 250 ng/mL for 2 days. The controls were ds*GFP*-injected whiteflies fed with 30% (w/v) sucrose solution. Then, bacteriocytes were dissected for localization of *Portiera* in bacteriocytes of female adult whiteflies by FISH following the protocol as described above. In addition, bacteriocytes were dissected for examination of PanBC level by immunofluorescence microscopy as described above. Three biological replicates were conducted.

To examine whether pantothenate supplementation restores the survival of dsRNA-injected whiteflies, ~800 female adult whiteflies within 4–6 days after emergence were microinjected with ds*panBC* and ds*GFP*. After

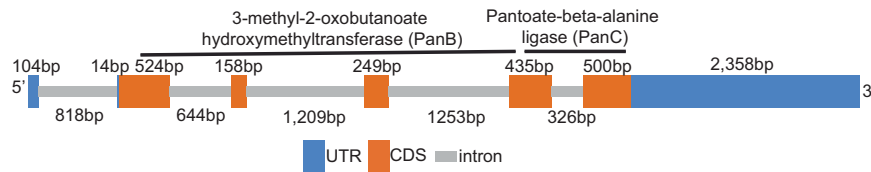**Fig. 1 Gene structure of *panBC* in *B. tabaci* MEAM1.** Gene structure of *panBC* was adapted from ref. [18] and the whitefly genome database (http://www.whiteflygenomics.org/cgi-bin/bta/geneinfo.cgi?&gene=Bta05339). The conserved domain presented is based on BLASTP results.

recovery on a cotton leaf disk for 12 h, 30 female adults per replicate of *panBC*-injected and ds*GFP*-injected whiteflies were fed 30% (w/v) sucrose solution supplemented with or without pantothenate at a final concentration of 250 ng/mL for 2 days. Then the mortality of injected female whiteflies was recorded. Six biological replicates were conducted.

To examine whether pantothenate supplementation restores the fecundity of dsRNA-injected whiteflies, dsRNA-injected whiteflies were fed 30% (w/v) sucrose solution supplemented with or without pantothenate at a final concentration of 250 ng/mL for 2 days as described above. Then, individual female adult whiteflies were released on cotton leaf disks. After 3 days, egg numbers were recorded. Ten replicates were conducted for each treatment.

Statistical analyses

For the OD values of the *E. coli* wild-type K-12, mutant K-12 and mutant K-12 transformants, statistical differences were evaluated using one-way ANOVA at a significance level of 0.05 followed by LSD post-hoc tests. For the symbiont titer, pantothenate amount, and mortality and egg numbers of ds*GFP*, and ds*panBC*-injected female whiteflies, statistical differences were evaluated using one-way ANOVA at a significance level of 0.05. Percentage data were transformed by arcsine square root before analysis. All data analyses were conducted using the STATISTICA v6.1 software (StatSoft, Inc., Tulsa, OK, USA).

Results

Prevalence of *panBC* in the bacterial symbiosis of whiteflies

To investigate whether *panBC* are ubiquitous in *B. tabaci*, the presence of *panBC* was determined in several *B. tabaci* species. We found that *panBC* is present in seven lab cultures of four *B. tabaci* species, which are distributed in Asia, America, and Africa (Supplementary Table 2). The *B. tabaci panBC* has five introns and five CDSs with two enzymatic functions [18] (Fig. 1 and Supplementary Fig. 1a). Thus, the domains corresponding to the bacterial PanB and PanC proteins as shown in Fig. 1 were analyzed

separately for sequence alignment and phylogenetic tree construction. To examine the divergence of protein sequences, amino acid sequences were aligned among three whitefly species, *B. aphidicola*, and *E. coli* for PanB and PanC. The amino acid sequence identity was high among all *B. tabaci* species (88.1% for PanB and 93.81% for PanC, respectively) but low among *B. tabaci*, *B. aphidicola*, and *E. coli* (68.91% for PanB and 72.36% for PanC, respectively) (Supplementary Fig. 1b, c). To gain insight into the evolution of *B. tabaci* PanBC, a phylogenetic tree was constructed. Interestingly, PanBC of all whitefly species clustered within the genus *Pseudomonas* (Supplementary Fig. 2). These data suggest that *panBC* has a common evolutionary origin in *B. tabaci*.

Functional complementation of *E. coli* pantothenate auxotrophs with whitefly *panBC*

To test the hypothesis that whitefly *panBC* functions in pantothenate synthesis, *E. coli* K-12 *panB* and *panC* knockout mutants ($-\Delta panB$ or $-\Delta panC$) were generated using the Lambda Red protocol and functionally complemented *E. coli* K-12 mutant with whitefly *panBC*, respectively. Compared to wild-type *E. coli*, *E. coli* K-12 knockout mutants ($-\Delta panB$ and $-\Delta panC$) did not grow on B5DP medium lacking pantothenate (Fig. 2). Although whitefly PanBC shared low amino acid sequence identities with *E. coli* homolog genes (41% and 42.57% for PanB and PanC, respectively) (Supplementary Fig. 1b, c), complementation with whitefly *panBC* rescued *E. coli* K-12 knockout mutants on B5DP medium (Fig. 2). In contrast, cells transformed with the pMD19-T empty vector did not grow on B5DP medium without pantothenate supplementation (Fig. 2). Significant differences in OD values among treatments were detected (Fig. 2; $F_{3,8} = 51.34$, $P < 0.0001$ for *panB* and $F_{3,8} = 50.09$, $P < 0.0001$ for *panC*).

Portiera elimination reduced pantothenate level, PanBC localization, and whitefly performance

To investigate whether *Portiera* contributes to the generation of pantothenate in whiteflies, *Portiera* were cured by antibiotic treatments. The *Portiera* titer was reduced by 97% and the titer of *Hamiltonella* and *Rickettisa* was reduced by 95–97%, by rifampicin treatment (Fig. 3A)
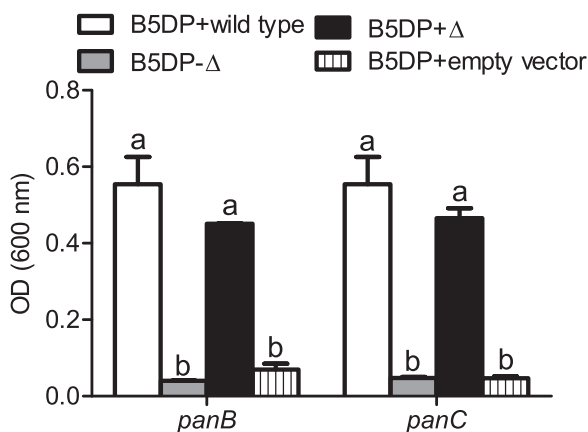
Fig. 2 Functional complementation of _E. coli_ pantothenate auxotrophs. _E. coli_ K-12 knockout mutant cells were transformed with expression plasmids containing whitefly _panBC_ or the negative control pMD19-T empty vector. The _E. coli_ wild-type K-12, mutant K-12 (−Δ), and mutant K-12 transformants (+Δ) were grown overnight in pantothenate-deficient B5DP medium at 37 °C. All _E. coli_ cells were washed and re-suspended to measure cell density at OD_{600}. Data are means ± SE. Different letters above the bars indicate significant differences between treatments at $P < 0.05$.

($F_{1,14} = 68.1$, $P < 0.0001$ for _Portiera_; $F_{1,14} = 43.77$, $P < 0.0001$ for _Hamiltonella_ and $F_{1,14} = 12.06$, $P = 0.0037$ for _Rickettsia_). The pantothenate level was decreased significantly in _Portiera_-cured (−PBt) compared to _Portiera_-infected (+PBt) whiteflies (Fig. 3B; $F_{1,4} = 14.37$, $P = 0.019$).

To test whether _Portiera_ influences expression of PanBC, we examined the subcellular location of the whitefly PanBC protein in bacteriocytes. The polyclonal antibody against PanBC protein was produced using the purified recombinant protein (Supplementary Fig. 3A–C). Immunofluorescence microscopy showed that PanBC has a high expression level in whitefly bacteriocytes. PanBC was located in the cytoplasmic region of bacteriocytes and had some clusters (Fig. 3C). Confocal microscopy observation confirmed that _Portiera_ was completely depleted in bacteriocytes after antibiotic treatments (Fig. 3D). After _Portiera_ was cured, the PanBC protein level decreased significantly and the PanBC protein localization pattern was altered with less protein clusters in whiteflies (Fig. 3D). These data suggest that the abundance of _Portiera_ impacts the pantothenate level and the localization of whitefly PanBC.

To test whether _Portiera_ affects whitefly performance, we examined whitefly survival and fecundity after _Portiera_ was cured. _Portiera_ elimination decreased the survival of female adult whiteflies (Fig. 4E; $F_{1,6} = 4.21$, $P = 0.086$ for day 3 and $F_{1,6} = 3.0$, $P = 0.13$ for day 6) and significantly reduced the whitefly fecundity (Fig. 4F; $F_{1,18} = 43.94$, $P < 0.0001$).

Silencing _panBC_ reduces symbiont titer, pantothenate level, and whitefly performance

To study the metabolic function of horizontally transferred _panBC_, whitefly _panBC_ was silenced by microinjection with ds_panBC_. The level of PanBC was significantly reduced in bacteriocytes at day 1, 3, and 5 after RNAi treatment but recovered at day 7 after microinjection with ds_panBC_ (Fig. 4A). In parallel with the changes in PanBC level, the titer of _Portiera_ also decreased in bacteriocytes at day 1, 3, and 5 but recovered at day 7 after RNAi treatment (Fig. 4A), as shown by FISH observation. _panBC_ gene silencing in whitefly bacteriocytes may not persist for more than 7 days after microinjection with dsRNA, which could lead to the recovery of PanCB and _Portiera_ levels at day 7. A qPCR test also showed that the titer of _Portiera_ was significantly decreased at day 3 after RNAi treatment (Fig. 4B; $F_{1,18} = 4.6$, $P = 0.046$). In contrast, the titer of _Hamiltonella_ remained unchanged at day 3 after RNAi treatment (Fig. 4B; $F_{1,18} = 0.04$, $P = 0.85$). As a result of gene silencing, the pantothenate level was decreased significantly in ds_panBC_-injected compared to ds_GFP_-injected whiteflies (Fig. 4C; $F_{1,4} = 12.45–13.42$, $P = 0.022–0.024$). In addition, silencing the horizontally transferred _panBC_ gene significantly increased the mortality of female adult whiteflies at days 3–7 after microinjection with dsRNAs (Fig. 4D; $F_{1,8} = 10.62–28.39$, $P = 0.0007–0.012$) and reduced female fecundity at day 7 after microinjection with dsRNAs (Fig. 4E; $F_{1,24} = 4.67$, $P = 0.041$).

Supplementation with pantothenate restores symbiont titer, PanBC level, and fitness of RNAi whiteflies

To examine whether B5 pantothenate supplementation can alter the titer of _Portiera_, PanBC level, and fitness of dsRNA-injected whiteflies, dsRNA-injected whiteflies were fed an artificial diet supplemented with pantothenate for 2 days. The titer of _Portiera_ increased in bacteriocytes of ds_panBC_-injected whiteflies supplemented with pantothenate, which was similar to ds_GFP_-injected whiteflies (Fig. 5A). The level of PanBC also recovered in bacteriocytes of RNAi whiteflies after pantothenate supplementation treatment (Fig. 5B). The mortality of ds_panBC_-injected whiteflies was increased significantly compared to ds_GFP_-injected whiteflies; after pantothenate supplementation, the mortality of ds_panBC_-injected whiteflies was decreased, which is close to that of ds_GFP_-injected whiteflies, while the mortality of ds_GFP_-injected whiteflies supplemented with pantothenate decreased but not significantly compared to ds_GFP_-injected whiteflies without pantothenate supplementation (Fig. 5C; $F_{5,18} = 102.15$, $P < 0.0001$). The fecundity of ds_panBC_-injected whiteflies was
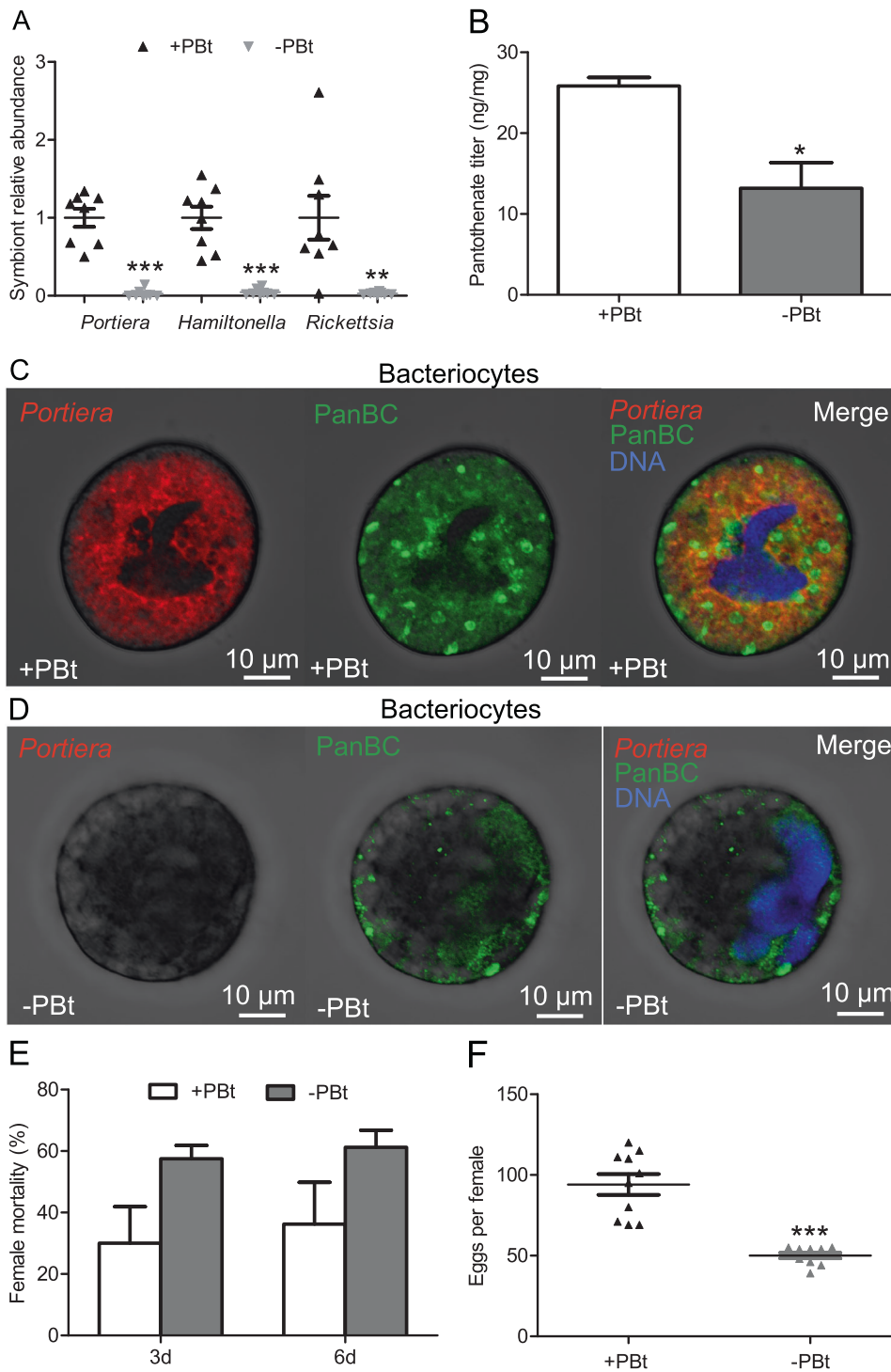
Fig. 3 Effect of *Portiera* elimination on pantothenate titer, PanBC level, and whitefly performance. A Effects of antibiotic treatments on the abundance of symbionts in *B. tabaci*. **B** Effects of *Portiera* elimination on pantothenate levels in *B. tabaci*. +PBt and −PBt represent *Portiera*-infected and *Portiera*-cured whiteflies, respectively. Localization of *Portiera* (red) and PanBC (green) in the bacteriocytes of +PBt (**C**) and −PBt (**D**) female adult whiteflies. DNA was stained with DAPI. **E** Mortality of +PBt and −PBt female adult whiteflies after feeding on cotton leaf disks for 3 days. **F** Fecundity of +PBt and −PBt female adult whiteflies after feeding on cotton leaf disks for 6 days. Data are means ± SE. The significant differences between treatments are indicated by asterisks in **A, B**, and **F** (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
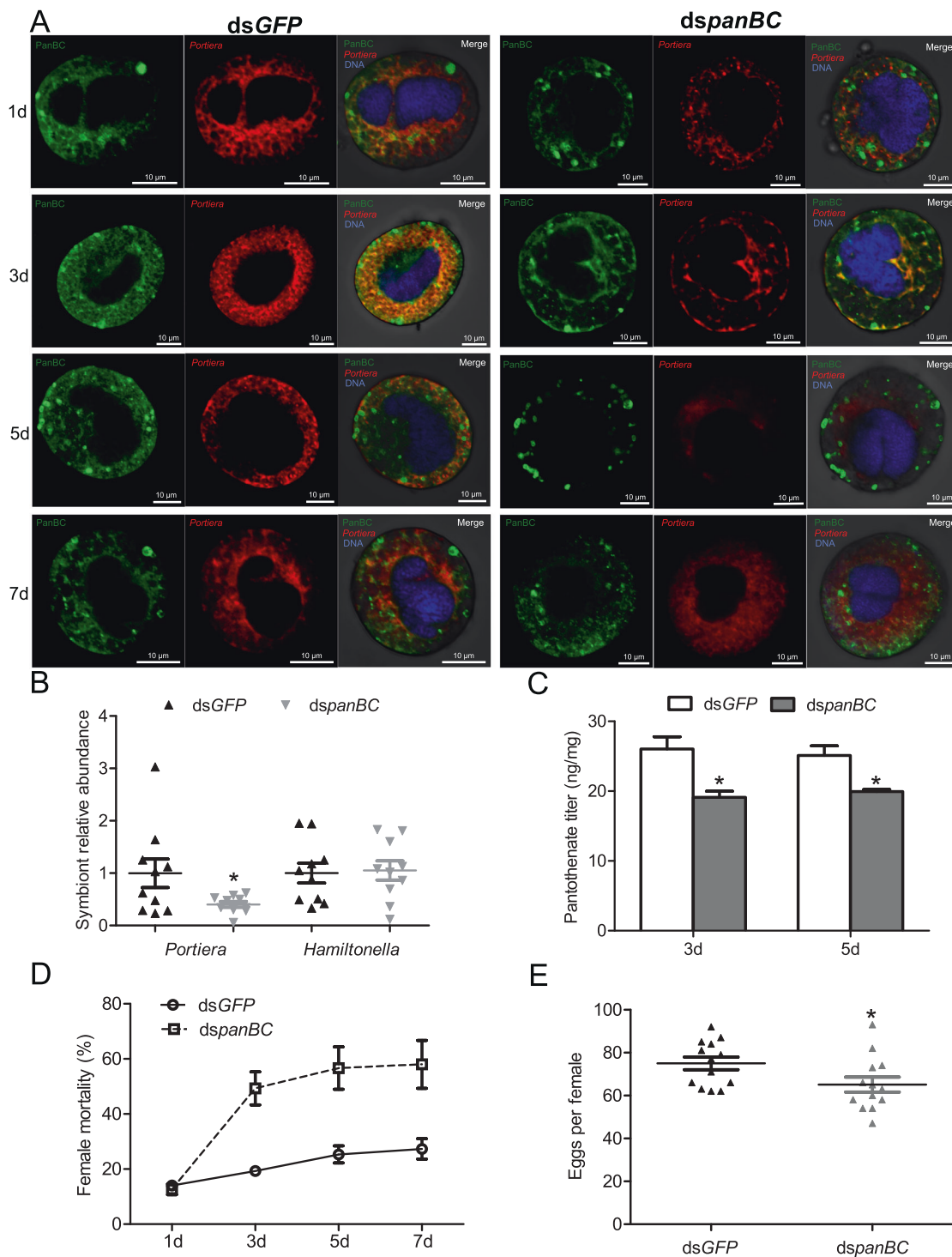
Fig. 4 Effects of silencing horizontally transferred *panBC* on PanBC localization, symbiont titer, pantothenate levels, and whitefly performance. A Localization of PanBC (green) and *Portiera* (red) in bacteriocytes of ds*GFP*-injected and ds*panBC*-injected female adult whiteflies at 1, 3, 5, and 7 days after whiteflies were micro-injected with dsRNA. DNA was stained with DAPI. **B** The symbiont titer in whiteflies at day 3 after whiteflies were microinjected with ds*panBC*. **C** Pantothenate levels in whiteflies at 3 and 5 days after whiteflies were microinjected with ds*panBC*. **D** Female mortality at 1, 3, 5, and 7 days after whiteflies was microinjected with ds*panBC*. **E** Female fecundity at day 7 after whiteflies was microinjected with ds*panBC*. Data are means ± SE. The significant differences between treatments are indicated by asterisks in **B**, **C**, and **E** (*$P < 0.05$).
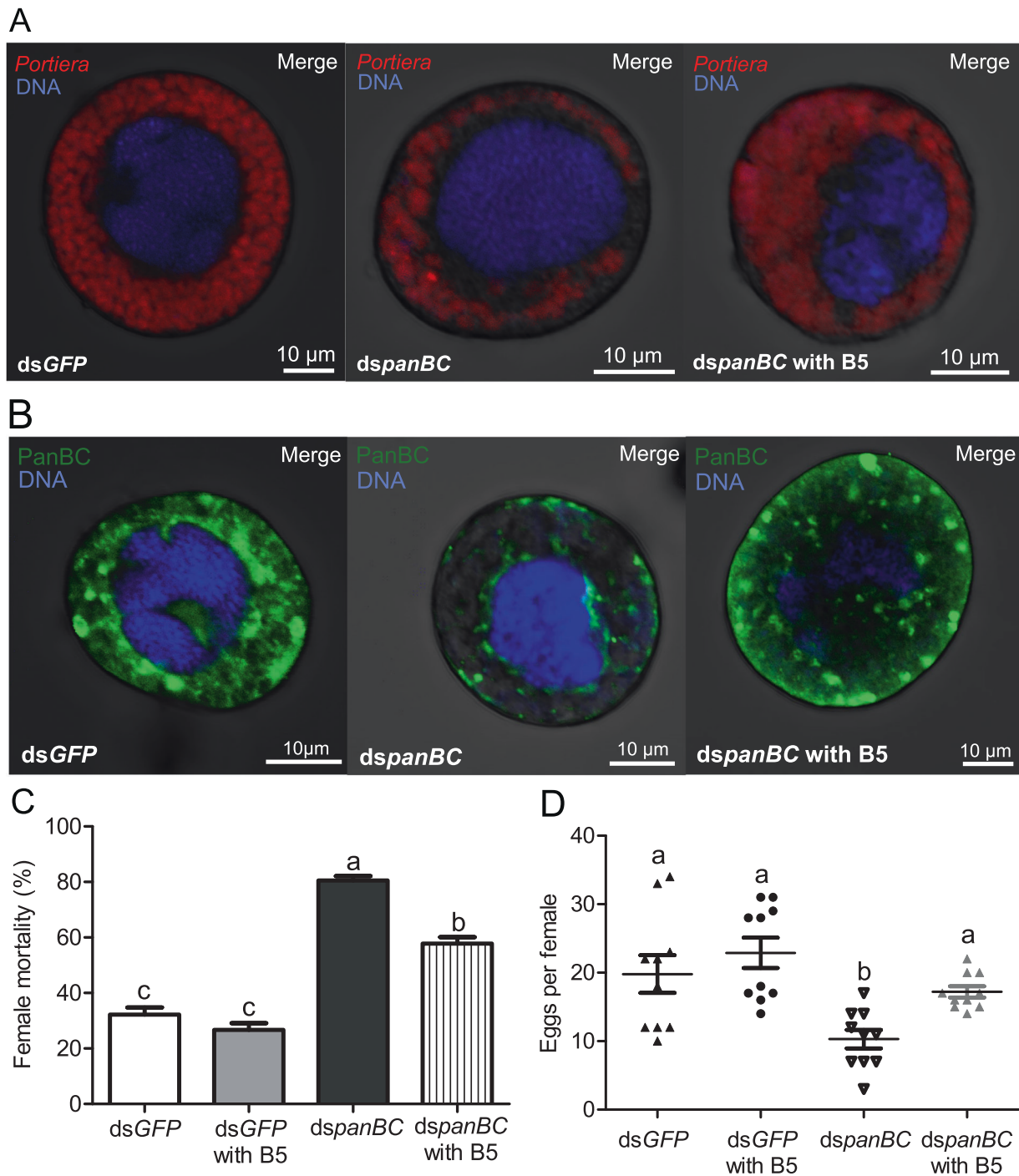
Fig. 5 Supplementation with pantothenate restores symbiont titer, PanBC level, and fitness of RNAi whiteflies. A Localization of *Portiera* (red) in bacteriocytes of ds*GFP*-injected and ds*panBC*-injected female adult whiteflies feeding on the artificial diet supplemented with or without B5 pantothenate for 2 days. DNA was stained with DAPI. **B** Localization of PanBC (green) in bacteriocytes of ds*GFP*-injected and ds*panBC*-injected female adult whiteflies feeding on the artificial diet supplemented with or without B5 pantothenate for 2 days. DNA was stained with DAPI. **C** Mortality of ds*GFP*-injected and ds*panBC*-injected female adult whiteflies after feeding on artificial diet supplemented with B5 pantothenate for 2 days. **D** Fecundity of ds*GFP*-injected and ds*panBC*-injected female adult whiteflies after feeding on artificial diet supplemented with B5 pantothenate for 2 days and then feeding on cotton leaf disk for 3 days. Data are means ± SE. Different letters above the bars indicate significant differences between treatments at $P < 0.05$.

decreased significantly compared to ds*GFP*-injected whiteflies; after pantothenate supplementation, the fecundity of ds*panBC*-injected whiteflies was restored to a level comparable to that of ds*GFP*-injected whiteflies, while the fecundity of ds*GFP*-injected whiteflies supplemented with pantothenate increased but not significantly compared to

ds*GFP*-injected whiteflies without pantothenate supplementation (Fig. [5]D; $F_{3,36} = 7.72$, $P = 0.00042$).

Discussion

Host adaptations are critical in enabling extreme gene loss of symbionts [1]. Insect genes can compensate for missing genes in symbionts [1, 3, 9, 10, 46, 47]. However, the regulation of insect bacterial symbiosis is not well known. We found co-regulation of whitefly fitness (PanBC protein level and whitefly performance) and symbiont titer. This co-regulation was mediated by the pantothenate level. Therefore, we revealed that a fused gene of bacterial origin can cooperate with *Portiera* to synthesize pantothenate. This collaboration facilitates the regulation of whitefly and symbiont fitness. Our findings demonstrate the key role of the horizontally transferred *panBC* gene in whitefly symbiosis.

The host–symbiont relationship is complex and dynamic [48]. System-level in silico analysis suggests that the biosynthesis of essential amino acids by *Buchnera* and *Portiera* is exclusively controlled by the aphid and whitefly host [49, 50]. The relative uniformity in the location and numbers of resident microbes also indicates that the insect host exerts tight control over its symbionts [51]. Aphids use the amino acid transporter to regulate the precursor glutamine supply to the bacteriocyte, thereby regulating amino acid biosynthesis in *Buchnera* [52]. Although the aphid seems to play the key role in regulating the metabolic output of bacteriocytes, *Buchnera* can regulate its gene expression by small RNAs [53, 54]. The host and symbiont gene expression between aphids and *Buchnera* appears to be coordinated [48]. We found co-regulation of whitefly PanBC protein abundance, whitefly performance, and symbiont titer. *Portiera* elimination or *panBC* silencing reduced the pantothenate level. Pantothenate is a vitamin (i.e. coenzyme) and the precursor of coenzyme A, which is required for the function of key enzymes in central carbon metabolism, especially involved in the TCA cycle and fatty acid metabolism [14]. The reduced pantothenate level likely inhibits the generation of lipids or other metabolites, which may repress protein synthesis, whitefly fecundity and survival, and symbiont proliferation. In contrast, the additional pantothenate allows *Porteria* to grow to high enough levels so that it may stimulate production of PanBC or directly facilitate PanBC synthesis and whitefly performance, regardless of RNAi treatment. Therefore, whitefly fitness (PanBC protein level and whitefly performance) and *Portiera* abundance can be co-regulated by the pantothenate level. This is supported by evidence that dietary supplementation with pantothenate restored *Portiera* abundance and whitefly PanBC protein level and whitefly performance.

The coordination of whitefly and symbiont fitness reflects the coadaptation and coevolution between host and its symbiont. The pantothenate-mediated coordination of whitefly and symbiont fitness suggests that the host and symbiont interactions are also modulated by nutrition status. These findings explain why whitefly fitness and symbiont abundance can vary among whitefly lineages. Furthermore, this study proposes that the regulation of metabolite synthesis in insect symbiosis may depend on the enzyme type, metabolite function, metabolite synthesis pathway, and the species involved. It extends our understanding the basis of complex host–symbiont interactions.

All of the four representative sternorrhynchan insect–bacterial symbiosis systems have distinct pantothenate synthesis pathways. The primary smbionts *Buchnera* of the aphid and *Moranella* of the mealybug retain the intact pantothenate synthesis pathway [3, 14, 16]. In contrast, the primary symbiont *Carsonella* of psyllids lacks *panBC*, which can be complemented by secondary symbionts in psyllids [9, 19]. The primary symbiont *Portiera* of *B. tabaci* only retains incomplete synthesis pathways for a few B vitamins including pantothenate. A fused gene *panBC* horizontally transferred from bacteria in whiteflies can complement for the missing gene in *Portiera* [18]. This result indicates the importance of pantothenate synthesis in insect symbiosis systems. All seven lab cultures of the four *B. tabaci* species harbored horizontally transferred *panBC* with a common evolutionary origin of *Pseudomonas*, which is absent in the *B. tabaci* species complex. Therefore, *panB* and *panC* were likely co-transferred to the common ancestor of *B. tabaci* and then later fused into the gene *panBC*. We demonstrated that whiteflies are able to synthesize pantothenate via horizontally transferred *panBC*. Because pantothenate is essential for whitefly fitness and *panBC* is widespread in *B. tabaci*, these results show that *panBC* contributes to the ability of whiteflies to feed on plant phloem deficient in B vitamins. Our findings suggest that pantothenate provisioning in animal symbiosis evolutionarily transitioned from bacterial symbionts to animal hosts through horizontal gene transfer events.

Rifampicin treatment leds to elimination of *Portiera*, *Hamiltonella*, and *Rickettisa* in *B. tabaci* MEAM1. Because *Hamiltonella* and *Rickettisa* have no pantothenate synthesis pathway [18], the reduced titer of *Hamiltonella* and *Rickettisa* could not have effects on pantothenate levels in *B. tabaci* MEAM1. *B. tabaci* MED harbors *Portiera* and *Hamiltonella*. After *Portiera* and *Hamiltonella* were cured in *B. tabaci* MED by rifampicin treatment, the survival rate and fecundity were also significantly reduced as that in *Portiera*, *Hamiltonella*, and *Rickettisa*-depleted *B. tabaci* MEAM1 [43, 44]. So *Rickettisa* elimination may play a minor role in the altered mortality rate and fecundity of symbionts-depleted *B. tabaci* MEAM1. Furthermore, our

previous studies demonstrated that *Hamiltonella* elimination only influenced the sex ratio and didn't affect mortality rate and fecundity of *B. tabaci* MEAM1 [15, 28]. Thus, it is an elimination of *Portiera* but not other symbionts that impacts the mortality rate and fecundity in *B. tabaci* MEAM1. Overall, *Portiera* elimination reduced the pantothenate level, thereby repressing the survival rate and fecundity of *B. tabaci* MEAM1.

PanBC has a high expression level in whitefly bacteriocytes. Both PanBC and *Portiera* cells were located in the cytoplasmic region of bacteriocytes. The high expression level and bacteriocyte localization of whitefly PanBC facilitates its cooperation with *Portiera* to synthesize pantothenate in *B. tabaci* MEAM1. In this case, pantothenate is produced in the cytoplasmic region of bacteriocytes, which may benefit its utilization by *Portiera* cells. Therefore, *Portiera* does not need the pantothenate symporter, which helps explain why it is lacking. In contrast, *Hamiltonella* and *Rickettisa* have totally lost the capability for pantothenate synthesis. However, they have pantothenate symporters to transport pantothenate into the cells of *Hamiltonella* or *Rickettisa* from the bacteriocytes where the whitefly and *Portiera* cooperate to synthesize this metabolite. There is lower titer of *Hamiltonella* in whiteflies as compared to *Portiera* [15, 27, 30], and changes of pantothenate levels may not have a large effect on the titer of *Hamiltonella* over short time periods. So we did not observe changes of pantothenate levels influencing the titer of *Hamiltonella* over 3 days after silencing *panBC*.

Acquisition of HTGs in the insect genome confers novel genetic information. Intron gain of HTGs is a critical step for them to attain functionality [15, 55]. In other species, *panB* and *panC* are separately present in the genome. However, the *B. tabaci panBC* has become a single gene and acquired introns [18]. This reveals that another way for HTGs to become functional is intron gain and further or simultaneous gene fusion or gene fusion and further intron gain. The PanB and PanC domains of whitefly PanBC mediate the proximal and final reactions in pantothenate synthesis [18]. Fused *panBC* in *B. tabaci* could make the chemical reaction more efficient during pantothenate production. In addition, the case of PanBC helps us to better understand the mechanisms underlying the formation of multiple domains of proteins.

Data availability

All relevant data supporting the findings of this study are included within the article and its Supplementary Information files.

Compliance with ethical standards

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