SUPPLEMENTARY MATERIAL

Efficient control of the fungal pathogens *Colletotrichum gloeosporioides* and *Penicillium digitatum* infecting citrus fruits by native soilborne *Bacillus velezensis* strains

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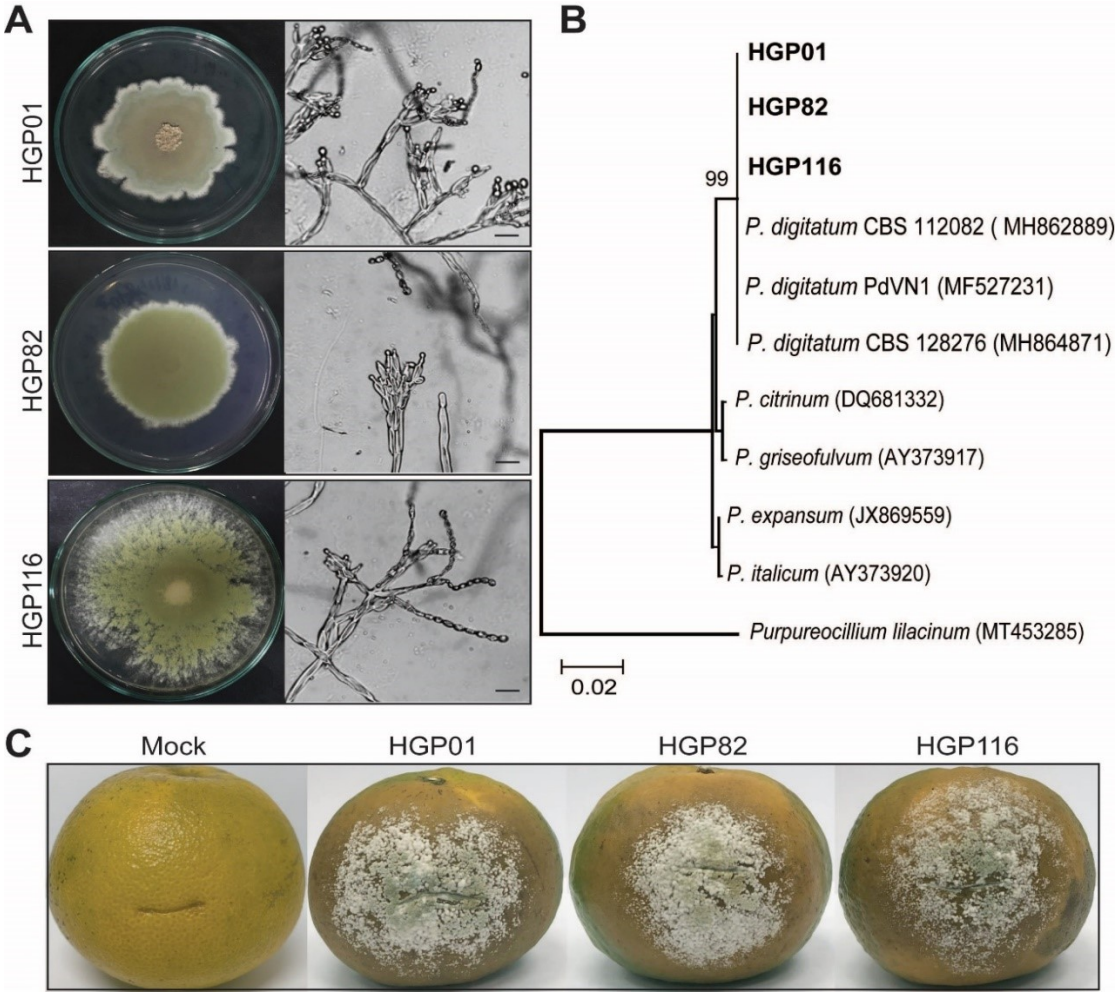

Figure S1. Identification of the citrus postharvest pathogen *P. digitatum*. (**A**) Morphological characteristics of three selected *P. digitatum* strains. (**B**) Molecular identification of these strains by ITS sequencing. The ITS sequences were compared to the GenBank database using the BLAST tool. The phylogenetic tree was constructed with MEGA X using the neighbor-joining method and 1000 bootstrap replications. Statistical support values at branch nodes of the tree, genetic distance scale, and accession numbers for the related ITS sequences extracted from GenBank are indicated. The ITS sequences for three *P. digitatum* strains (HGP01, HGP82, HGP116) are deposited in GenBank as the accession numbers OL739239–OL739241. (**C**) Reinfection of three isolated strains on orange fruits to confirm their pathogenicity. Fungal spore suspensions (10^6 spores/mL) were utilized for the infection assays. Negative control fruits (mock) were inoculated with sterile distilled water. The fruits were incubated in sterile plastic boxes for 7 days. Scale bars indicate the sizes of the images.

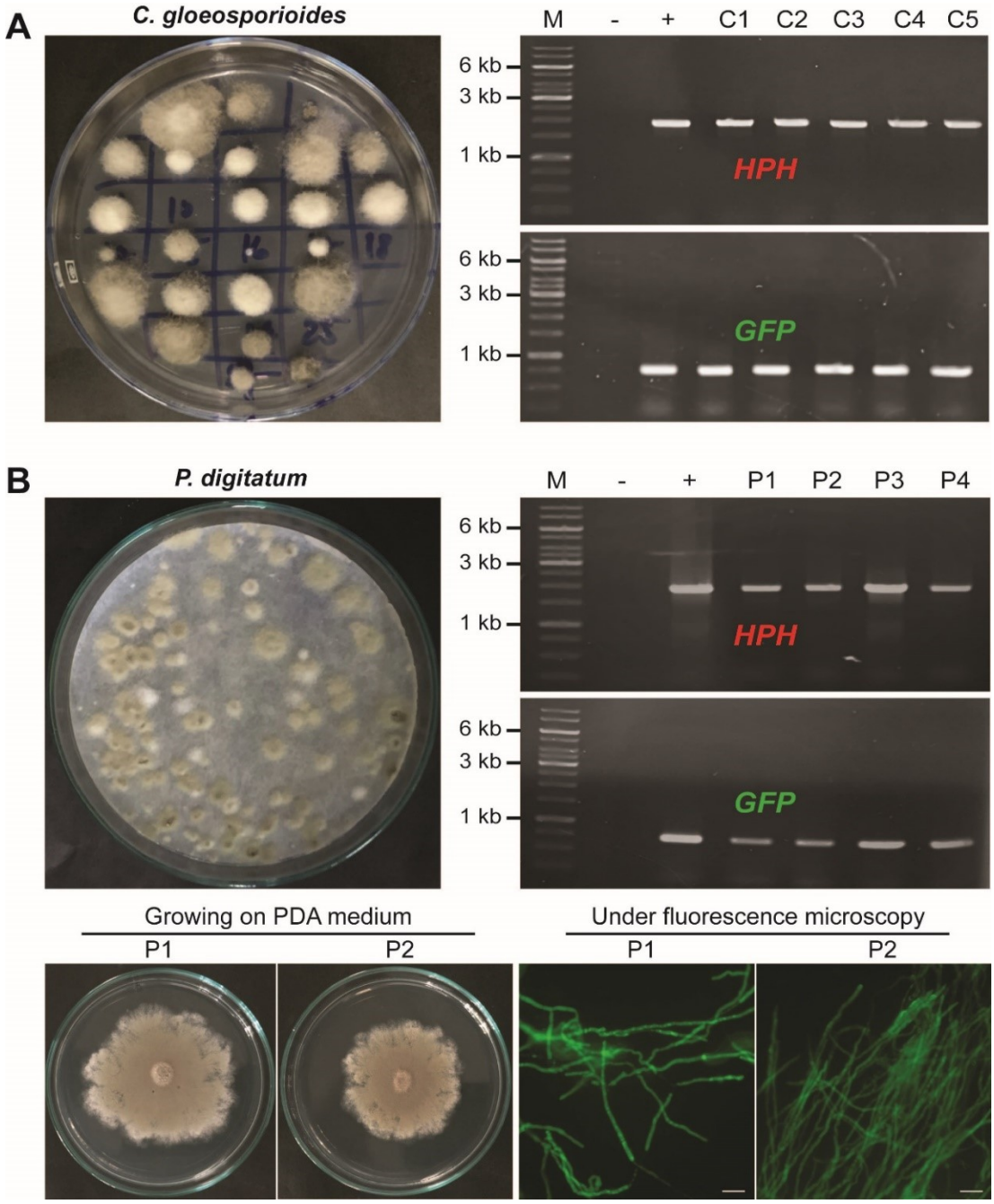

Figure S2. The integration of the *GFP* expression cassette into genomes of the citrus fungal pathogens using the ATMT method. The transformants of *C. gloeosporioides* (**A**) and *P. digitatum* (**B**) were grown on the PDA medium supplemented with hygromycin (200–300 μg/mL). Some transformants were selected for total DNA extraction. The presence of the hygromycin resistance gene (*HPH*) and the *GFP* reporter gene in their genomes was detected by PCR using the specific primer pairs HPH-F/HPH-R and GFP-F/GFP-R. Two different transformants (P1, P2) generated from *P. digitatum* HGP01 were confirmed for the *GFP* expression under fluorescence microscopy. Scale bars indicate the sizes of the images.

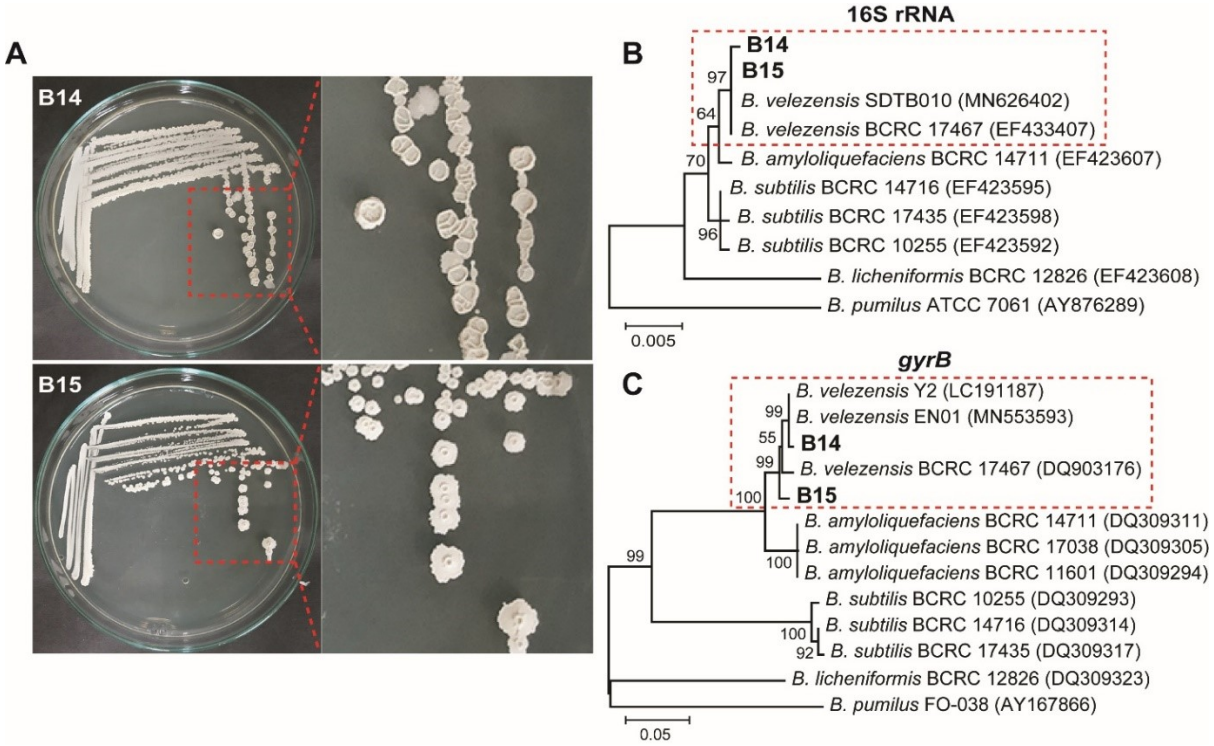

Figure S3. Identification of the antagonistic *Bacillus* isolates. Colonies of the *Bacillus* strains B14 and B15 on the LB plates after 2 days of cultivation at 30 ºC (**A**). Total DNA samples from the pure cultures of these bacterial strains were used for PCR amplification and sequencing analyses of two different genes, including 16S rRNA (**B**) and *gyrB* (**C**). Phylogenetic trees were constructed with MEGA X using the neighbor-joining statistical method and 1000 bootstrap replications. Statistical support values at branch nodes of the trees, genetic distance scale, and accession numbers for the homologous sequences extracted from GenBank are indicated. The sequences for 16S rRNA and *gyrB* genes from two *B. velezensis* isolates (B14, B15) are deposited in GenBank as the accession numbers OL706755–OL706756 and OL782197–OL782198, respectively.

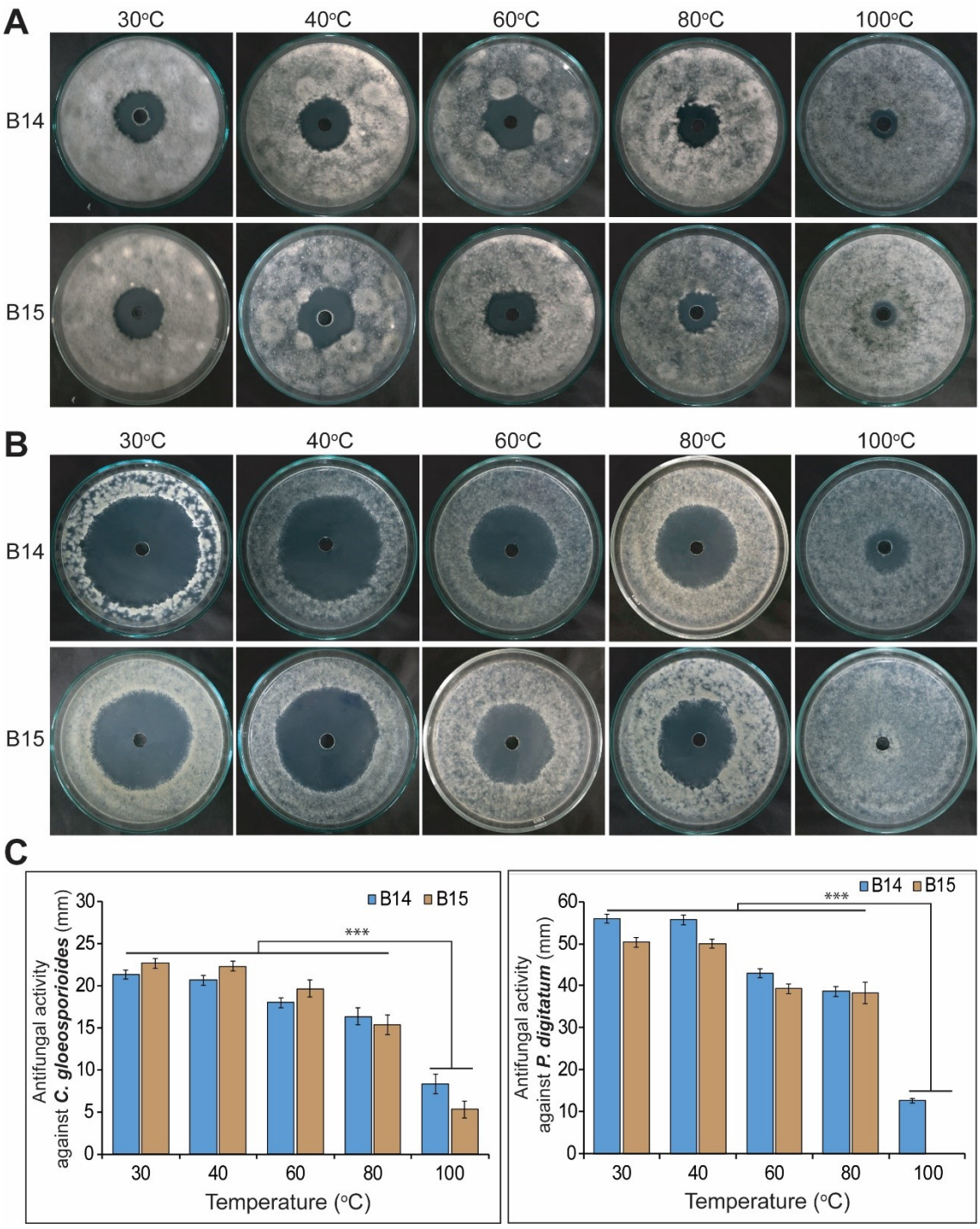

Figure S4. Antifungal activity assays of culture supernatants for thermostability. The supernatants from bacterial cultures of B14 and B15 were treated at different temperatures (30–100 ºC) using a block heater. Antifungal activity of the heat-treated supernatants against *C. gloeosporioides* (**A**) and *P. digitatum* (**B**) was evaluated with the agar well diffusion method, and the diameters of inhibition zones were measured (**C**). The experiments were performed in triplicate, and data were expressed as mean ± SD. The asterisk (***) indicates a statistically significant difference ($p < 0.001$) when comparing the antifungal activity of the supernatants treated at lower temperatures (30–80ºC) with that of the supernatants treated at 100ºC.

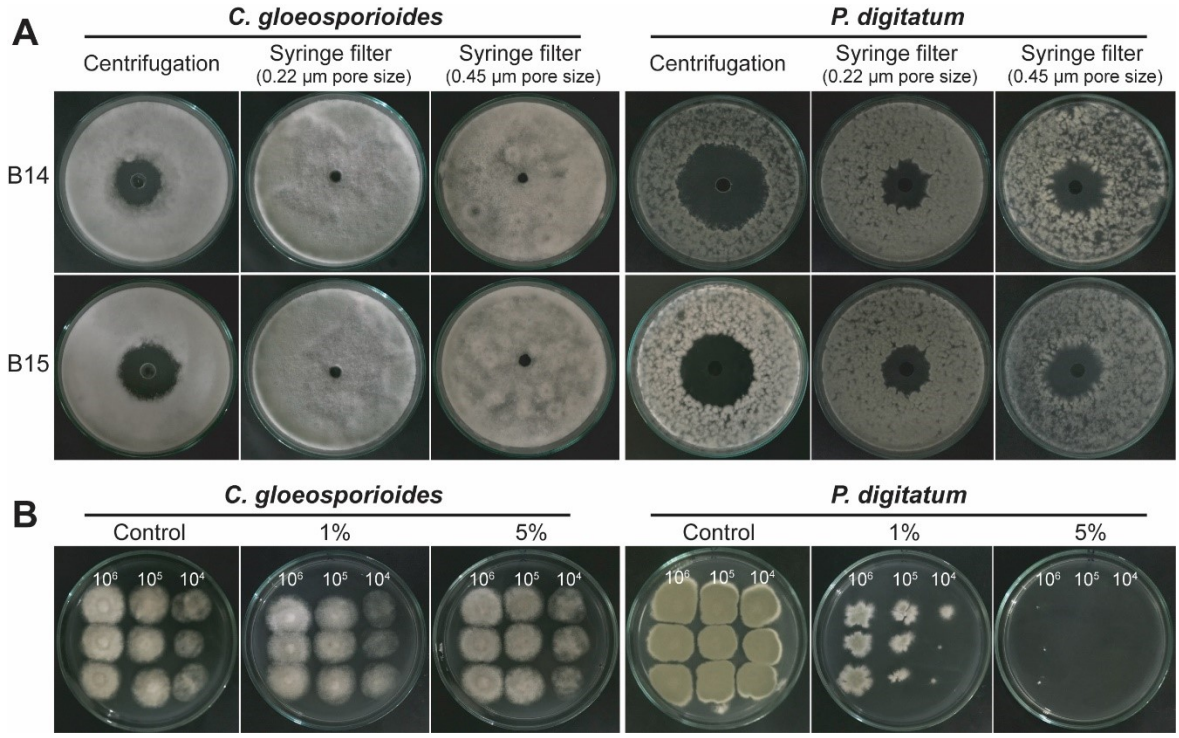

Figure S5. Antifungal activity assays of bacterial culture filtrates against *C. gloeosporioides* and *P. digitatum*. The bacterial cultures were filtered through sterile syringe filters with a pore size of 0.22 μm or 0.45 μm to obtain cell-free filtrates. (**A**) The filtrates were used for agar well diffusion assays. The PDA plates were entirely covered with fungal spores from *C. gloeosporioides* or *P. digitatum*. Filtrate volumes of 30 μL were added to agar wells in the center of the plates Culture supernatants from centrifugation were used as positive controls for comparison. (**B**) Antagonistic activity of the filtrates was examined by adding 1 % or 5% (v/v) of each filtrate to the PDA medium, and volumes of 10 μL of diluted spore suspensions (10^4, 10^5, 10^6 spores/mL) were inoculated onto the surface of the PDA plates. The plates were incubated at 25 ºC for 5 days.

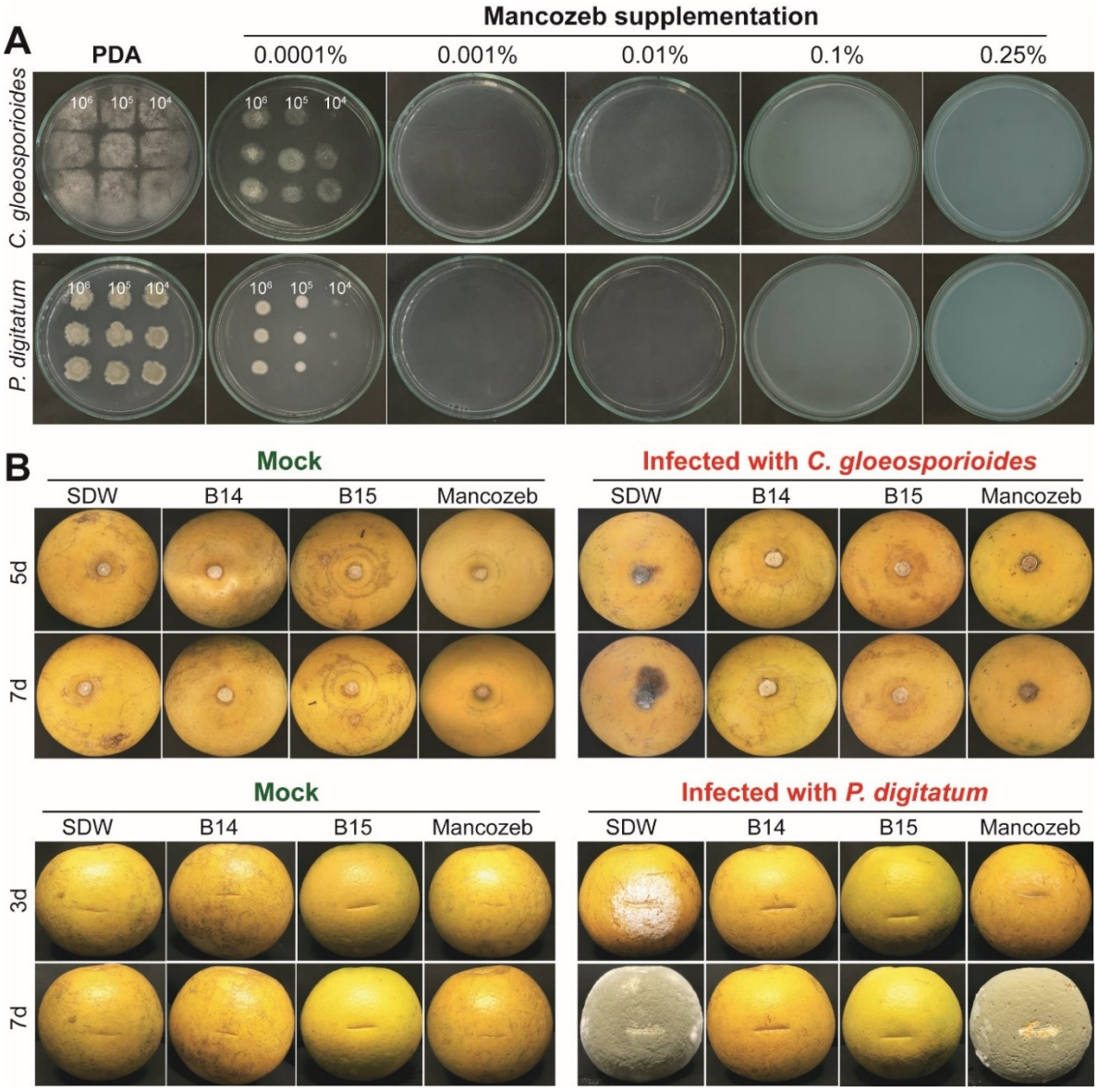

Figure S6. A comparison of antifungal ability between the *B. velezensis* strains and the synthetic fungicide mancozeb. (**A**) The antifungal ability of mancozeb was examined at different concentrations. The fungicide was added to the PDA medium to achieve a final concentration of 0.0001–0.25%. Volumes of 10 μL of diluted spore suspensions (10^4, 10^5, 10^6 spores/mL) were spotted on the PDA plates. The plates were incubated at 25 ºC for 5 days. (**B**) *In vivo* assays of the antifungal ability of the *B. velezensis* strains (B14 and B15) in protecting citrus fruits. Orange fruits were soaked in bacterial cultures or mancozeb (0.25%) for 30 min and air-dried at room temperature. The fruits were artificially wounded and infected with fungal spores from *C. gloeosporioides* HGC201 or *P. digitatum* HGP01. Negative control fruits (mock) were inoculated with SDW. The infected fruits were incubated at 25 ºC for 3–7 days.