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ORIGINAL ARTICLE

Pantoea stewartii subsp. *stewartii*, the causative agent of Thai jackfruit's bronzing disease and its possible host range in Vietnam

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Abstract

The bronzing disease of Thai jackfruit (*Artocarpus heterophyllus* Lam.) has recently appeared in Vietnam, causing a significant loss for farmers, but its control method is still restricted. In this study, we identified pathogens based on morphological and molecular characterizations. A total of 25 bacterial isolates were isolated from diseased samples. The bacterium produces white or yellow pigment in culture, is gram-negative, slightly pleomorphic, non-motile, facultatively anaerobic, short-rod, and catalase-positive. It hydrolyzes gelatin and starch but not tween 80, and produces acid from glucose, sucrose, and lactose. The bacterium does not produce indole and does not produce hypersensitivity to tobacco. The results of sequencing of the encoded region of synthesis of capsular polysaccharide (*cpsD*) and pathogenicity-related genes *HrpS* confirmed that the causative agent is *Pantoea stewartii* subsp. *stewartii.* The pathogen's possible host range could be traditional jackfruit varieties, fruits, and crops such as durian, longan, mango, tomatoes, broccoli, pumpkin, cucumber, corn, rice, sweet potatoes, water spinach, peanuts, and green beans. The *P. stewartii* subsp. *stewartii* could enter the host plant cell through open wounds or natural openings such as stomata. The results confirmed the presence of *P. stewartii* subsp. *stewartii* in Vietnam and suggest that the jackfruit tree should not be planted in plantations with these crops to prevent cross-contamination and the spread of pathogens.

Keywords: bronzing of Thai jackfruit, causal agent, cross-infect, host range, *Pantoea stewartii* subsp. *stewartii*

Introduction

Bronzing of jackfruit disease was first reported in Central and South America in 2011, and since then, it has been reported in many countries, including the Philippines, Mexico, and Malaysia (Gapasin *et al.* 2014; Hernández-Morales *et al.* 2017; Zulperi *et al.* 2017). In Vietnam, the bronzing of Thai jackfruit occurred in 2016 and spread over all growing areas, including Tien Giang, Ho Chi Minh City, Dong Nai, Lam Dong, Hau Giang, Long An, and Dong Thap provinces, causing significant losses for farmers (Vo *et al.* 2023b).

Based on morphological characterizations and sequencing *cps* gene cluster, which encodes the region of synthesis of capsular polysaccharide, Vo *et al.* (2023a) announced that the causative agent of bronzing of Thai jackfruit in Ho Chi Minh City was *Pantoea stewartia* subsp. *stewartii* (*Pss*). However, the EPPO (EPPO 2009) reported the current absence of *Pss* in Vietnam, so its management faced difficulties.

Pss is well-known as Stewart's bacterial wilt or leaf blight of corn causative agent, and can infect rice (*Oryza sativa*), oats (*Avena sativa*), and common wheat (*Triticum aestivum*), as well as the ornamental *Dracaena sanderiana*, and palm *Bactris gasipaes* (Jeger *et al.* 2018). In addition, *Pss* has been reported as a causative agent of bacterial leaf wilt of sugarcane in China (Cui *et al.* 2021). The *Pss* pathogen isolated from bronzing

jackfruit in Malaysia could affect sweetcorn, pineapple, and cucumber *in vitro* (Abidin *et al.* 2021). In addition, other *Pantoea* species are severe pathogens of rice that cause diseases worldwide (Azizi *et al.* 2020), as well as on other crops such as strawberries (Abdel-Gaied *et al.* 2022) in the field and cabbage heads in storage (Eichmeier *et al.* 2017).

In Vietnam, jackfruit plantations are usually located near other production areas of tropical fruits and crops (Vo 2023b). It is essential to correctly identify the causative agent of jackfruit-bronzing in different regions and the possibility of cross-contamination with traditional jackfruit varieties and other fruits and crops to properly assess the host range and develop effective control measures.

Materials and Methods

Sample collection and isolation of bacterial pathogen

Jackfruit diseased samples of the Thai variety, 10 to 100 days after pollination, showing abnormal stalks, fruit ring color, fruit shape, and fruitlets as well as symptomless samples were collected from plantation areas in different southern provinces of Vietnam, including Lam Dong, Dong Nai, Ho Chi Minh City, Long An, and Tien Giang from 2020 to 2022. The infected fruits were wrapped in a napkin, put into sterile plastic bags, labeled, and taken to the laboratory for initial diagnosis and pathogen isolation within 24 hours. The pathogen isolation procedure was done as described by Gapasin (2014) using King's B agar medium. The pure bacterial cultures were stored in sterilized water under room conditions and at –80°C in 50% glycerol for later use.

Pathogenicity tests, characterization and identification of the pathogens

A pathogenicity test on Thai jackfruit was conducted using the method described by Abidin (2021). Bronzing symptoms were assessed daily for up to 2 weeks of inoculation. The pathogens were re-isolated from infected pulps and identified as identical to the initial bacteria to fulfill the required Koch postulates. The experiment was repeated three times.

The phenotypic characterization of each isolate that produced typical disease symptoms was determined via colony morphology, pigmentation, and biochemical testing according to Gapasin (2014) and Abidin (2021). A homogenous suspension of test organism with 10^8 cfu · ml^{-1} was prepared for the tobacco hypersensitivity test. With sterile water alone, *Escherichia coli* ATCC 35218 (negative control) provided by the Institute of Tropical Biology, Vietnam, and *Ralstonia solanacearum* strain *RS1* (positive control) were injected into the underside of tobacco leaves using a 1 ml syringe. All tests were performed using freshly grown colonies (24 h to 48 h).

Molecular characterization of pathogens was done based on sequencing of the encoding region of synthesis of capsular polysaccharide (*cpsD*) with primers CPSL1 (5'CCTGTCAGTCTCGAACC 3') and CPSR2c (5'ATCTCGAACCGGTAACC 3'), as well as primers HRP1d (GCACTCATTCCGACCAC) and HRP3c (GCGGCATACCTAACTCC) of pathogenicity-related genes *HrpS*. *HrpS* is a bacterial enhancer binding protein (bEBP) – *HrpS* type III transcriptional regulator (Abidin *et al.* 2020). All bacterial isolates were grown on liquid King'B medium for 24 to 48 h at 28°C and centrifuged at 150 rpm · min^{-1}. Using the provided protocol, a commercial genomic DNA isolation kit – TopPURE® Genomic DNA Extraction Kit (ABT Biotech LTD., Vietnam), was used to extract bacterial DNA. The polymerase chain reaction (PCR) was performed in a 25 µl reaction mixture, containing 2 µl DNA (1 ng · $µl^{-1}$) of extracted total genomic DNA template, 12.5 µl of 2x DreamTaq Red PCR MasterMix (Thermo Scientific Inc., USA), 6.5 µl of DNase-free water and 2 µl (20 pM) of each primer (Apical Scientific, Malaysia). PCR amplification was performed in an 'iCycler' Thermal Cycler (Bio-Rad Laboratories Inc., USA) according to the following protocol: Denaturation was done at 94°C for 5 min, 35 cycles (denaturation at 94°C for 30 s, annealing at 56°C for 30 s in the case of the *cpsD* primer and at 52°C for 30 s *HrpS* primers; and extension at 72°C for 30 s) and final extension at 72°C for 7 minutes. The PCR product was separated on a 1.5% agarose gel (40 minutes at 1.5 V · cm^{-1}), stained with 6X GelRed, and photographed under UV light. After that, PCR products were sequenced using Sanger technology. All sequences were analyzed using Bioedit software, and the similarity of these strains was checked with strains published in the National Center for Biotechnology Information (NCBI) database using BLAST search. The sequence alignments were analyzed to design the phylogenetic tree with a bootstrap coefficient 1000 on distance method using the maximum parsimony (MP) and neighbor-joining (NJ) in the software MEGA 11 (Kumar *et al.* 2018). The *cpsD* and *HrpS* sequences were submitted to GenBank database, accession numbers were assigned, and added to Table 1.

Host range testing on other jackfruit varieties and plants

The bacteria *Pss* strain HCM09 confirmed by PCR analysis presented above was used to inoculate other jackfruit varieties and possible host plants. Three traditional jackfruit varieties involved cv. Labang, cv.

Table 1. Summary of selected tests done on the bronzing bacterium

№	Isolates	Collection area	Time of collection	GS	KOH	M	CR	OR	IP	SH	T80	GL	HR	CT			Genbank no. cpsD	Genbank no. HrpS
														G	S	L		
1	LD01	11°36′36.4″N 108°04′50.0″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900442	OR100682
2	LD02	11°43′24.5″N 108°04′48.0″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900443	nd
3	LD03	11°39′11.8″N 107°17′45.9″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900444	nd
4	LD04	11°30′30.4″N 107°28′29.8″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900445	nd
5	DN01	10°55′56.8″N 107°16′09.6″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900435	OR100672
6	DN02	11°01′40.0″N 107°17′20.5″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900436	OR100673
7	DN03	11°22′56.5″N 107°23′44.2″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900437	nd
8	DN04	11°19′01.3″N 107°19′04.0″E	2021	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900438	OR100674
9	DN05	11°22′56.0″N 107°23′49.0″E	2021	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900439	OR100675
10	HCM01	11°01′17.8″N 106°33′26.7″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900426	nd
11	HCM02	11°00′47.0″N 106°31′47.0″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900427	nd
12	HCM03	11°00′47.0″N 106°31′47.0″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900428	nd
13	HCM04	10°59′30.0″N 106°36′01.5″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900429	nd
14	HCM05	10°59′30.0″N 106°36′01.5″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900430	OR100676
15	HCM06	11°00′49.8″N 106°35′13.0″E	2020	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900431	OR100677
16	HCM07	11°00′49.8″N 106°35′13.0″E	2021	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900432	OR100678
17	HCM08	11°08′29.2″N 106°28′14.6″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900433	OR100679
18	HCM09	11°06′34.0″N 106°29′27.7″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900434	OR100680
19	LA01	10°43′57.6″N 105°51′47.6″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900440	OR100681
20	LA02	10°38′56.5″N 106°10′22.4″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900441	nd
21	TG01	10°21′57.0″N 105°56′54.2″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900446	OR100683
22	TG02	10°21′43.5″N 105°56′42.7″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900447	OR100684
23	TG03	10°25′25.7″N 106°05′04.8″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900448	OR100685
24	TG04	10°32′22.6″N 106°11′48.9″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900449	OR100686
25	TG05	10°24′57.7″N 106°07′00.2″E	2022	–	–	–	+	–	–	+	–	+	–	+	+	+	OR900450	nd

LD – Lam Dong province, DN – Dong Nai province, HCM – Ho Chi Minh City, LA – Long An province, TG – Tien Giang province, GS – gram dye reaction, KOH – KOH test, M – potency motility, CR – catalase reaction, OR – Kovac's oxidase reaction, IP – indole reaction, SH – starch hydrolysis, T80 – Tween 80 hydrolysis, GL – gelatine degradation, HR – hypersensitivity to tobacco plant, CT – carbohydrate oxidation reaction, G – glucose, S – sucrose, L – lactose, (+) – positive reaction, (–) – negative reaction; nd – no data

Nghe, and cv. Tonu. Other fruits examined were mango (*Mangifera indica*), chili (*Dimocarpus longan*), durian (*Durio zibethinus*), and guava (*Psidium guajava*). Crops including tomato (*Solanum lycopersicum*), mustard (*Brassica juncea*), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*), corn (*Zea mays var. amylacea*), rice (*Oryza sativa*), sweet potato (*Ipomoea batatas*), water spinach (*Ipomoea aquatica*), and peanut (*Arachis hypogaea*) were used in this experiment. The leaves and pulps of traditional jackfruits and tropical fruits were collected at a reserved area of the Southern Horticultural Research Institute, Vietnam. The seeds of tomato, mustard, cucumber, pumpkin, corn, sweet potato, water spinach, and peanut were provided by the Southern Seed Corporation (SSC). The seeds of rice variety IR50401 were provided by the Loc Troi Group.

A pathogenicity test on traditional jackfruit varieties was performed using the method described for the pathogenicity test on Thai variety. For the remaining plants, 20 symptomless leaves of each plant were collected, sterilized by water and ethanol 70%, and put in a humidity box (30 × 25 × 13 cm) with a layer of sterile paper. Half of the leaves was infected by wound-mediated and nonwounded inoculation by adding 50 µl *Pss* suspension 10^6 cfu · ml^{-1} to the central vascular system. Cotton was used to maintain the bacterial suspension and removed 24 hours after injection. Fifty µl sterile water was injected separately and served as the control. All the boxes were held under room conditions (25°C ± 2°C) for 7 days, and when symptoms appeared on each leaf, disease incidence was calculated.

The greenhouse experiment used plants grown in pots (10 × 8 × 10 cm). When all plants had 4–6 true leaves, the bacterial suspension *Pss* strain HCM09 10^6 cfu · ml^{-1} was sprayed on all the plants evenly in the afternoon. Each plant was observed daily for symptoms and disease incidence was recorded 7 days after injection. During the inoculation experiment, the temperature in the greenhouse was 28°C ± 2°C, and the humidity was 75% ± 5% with 12 hours of light daily.

Results and Discussion

Isolation, biochemical characterization of pathogens, and pathogenicity tests

Field-collected jackfruits that had abnormal symptoms on the outside of the left stalk, altered fruit ring color or fruit shape, were fruitless or symptomless had been affected by bronzing and showed rusty, reddish discoloration in the pulp and rags. A total of 25 isolates was selected based on colony morphology and biochemical characterization. The suspected isolates were gram-negative bacteria, with white or yellow pigment in culture produced around the colonies on King's B agar medium. Bacteria were slightly pleomorphic nonmotile, facultatively anaerobic, and catalase positive. They hydrolyzed gelatin and starch, but not tween 80, did not produce indole, and produced acid from glucose, sucrose, and lactose. A summary of the critical tests towards its identification is shown in Table 1.

In the hypersensitivity test with necrosis on tobacco leaves (Fig. 1), all isolates showed a negative result, compared to a positive outcome of *R. solanacearum* because of the absence of the HR reaction 48 h after inoculation. These results were consistent with other published reports on the characteristics of *Pantoea* species strains related to jackfruit bronzing disease in the Philippines (Gapasin *et al.* 2014). However, in other reports the *Pss*

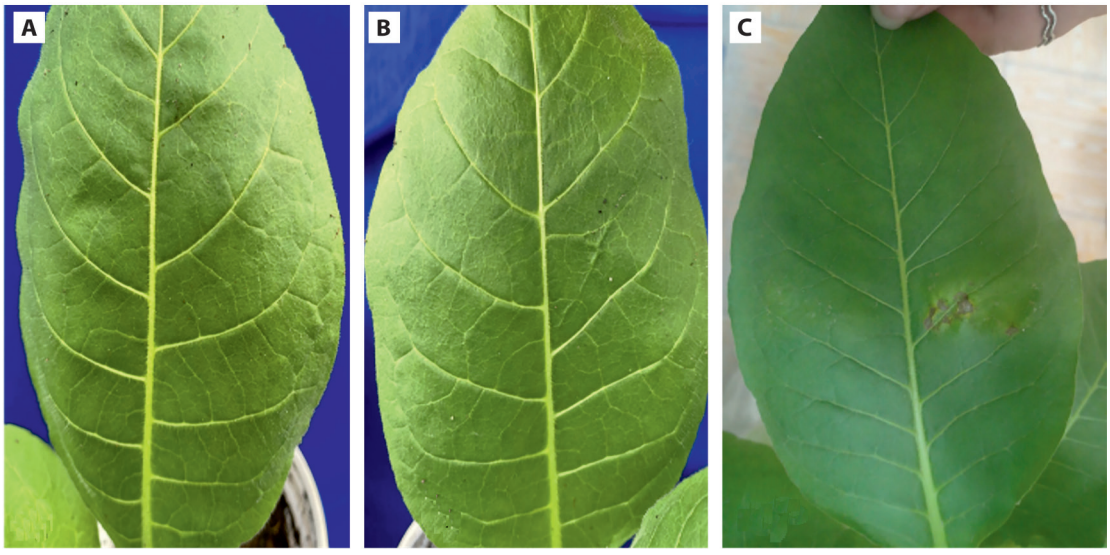

Fig. 1. Hypersensitivity test on tobacco leaves: A, B – symptomless inoculated by Pss strain HCM09 and *Escherichia coli*; C – inoculated by *Ralstonia solanacearum*

related to jackfruit in Malaysia can cause HR on tobacco (Abidin *et al.* 2020; Ibrahim *et al.* 2020) and showed the PCR success with *HrpS* amplification.

The pathogenicity test played an essential role in determining the disease's causative agents because the bronzing of jackfruit could appear with externally unclear symptoms. This study used two different inoculation methods to confirm the disease symptoms. Bacterial suspension of all isolates was used to inoculate the detached and attached fruits for 2 weeks, and the inoculated fruits were sliced to confirm the development of bronzing symptoms.

The bacterial isolates caused typical symptoms of reddish discoloration and rusty or bronzing specks with yellowish discoloration of Thai jackfruit pulps (Fig. 2B, C) which did not appear in the control (Fig. 2A, D). When the bacterial suspension was injected into the attached fruit, after 2 weeks, the inside of the fruits showed a yellow discoloration, which visibly started from the point of injection and spread into the base and to the rags (Fig. 2E).

The same symptoms were observed in the fruits infected by re-isolation bacteria. However, the bronzing symptoms of the inoculated fruits were lighter than those of the naturally infected fruits. The time limit could be because the inoculated fruits were opened 2 weeks after the injection. Bacteria only began to spread inside the fruit, and the infection was still at an early stage. Meanwhile, natural infection might have occurred earlier from fruiting (Vo 2023b), and the fruits could be infected, continuing to develop until ripening.

Molecular identification of the pathogens

In this study, 25 bacterial isolates were successfully amplified with primers CPSL1/ CPSR2c, PCR products ranging in size from 1100 bp to 1200 bp, and 14 bacterial isolates amplified with primers HRP1d/ HRP3c which PCR product was 900–1000 bp. PCR products were subjected to DNA sequencing using Sanger sequencing at Genlab Co., Ltd. The BLAST tool compared the obtained DNA sequences to known reference sequences in NCBI GenBank databases.

The investigated isolates had 97.64–100% similarity with *P. stewartii* subsp. *stewartii* (*Pss*) strain MS9 (accession MW971438.1) and *Pss* strain NWm6 (accession MH752487.1) with coverage ranging from 98 to 100%. Both of these strains have been identified as the cause of bronzing of jackfruit disease in Malaysia (Abidin *et al.* 2017; Ibrahim *et al.* 2020).

Bacteria *P. stewartii* subsp. *stewartii* strain MS9 (accession MW971438.1), strain NWj9 (accession MH752481.1), strain NWm6 (accession MH752487.1), strain I10 (accession MW971431.1), strain M8 (MW971434.1) and strain W15 (accession MH257290.1) cause disease on jackfruit; strain DC283 (accession CP017581.1), strain ICMP (accession AB894429.1) and strain DOAB 022 (accession EU215384.1) cause disease on maize; strain AG024-VI (accession MW115978.1) causes disease on beans; *P. agglomerans* strain Hcar02a (accession JQ081302.1) as an outgroup was used to construct the phylogenetic tree (Fig. 3A). This phylogenetic tree has the highest bootstrap rate of 100% and the lowest rate of 8%.

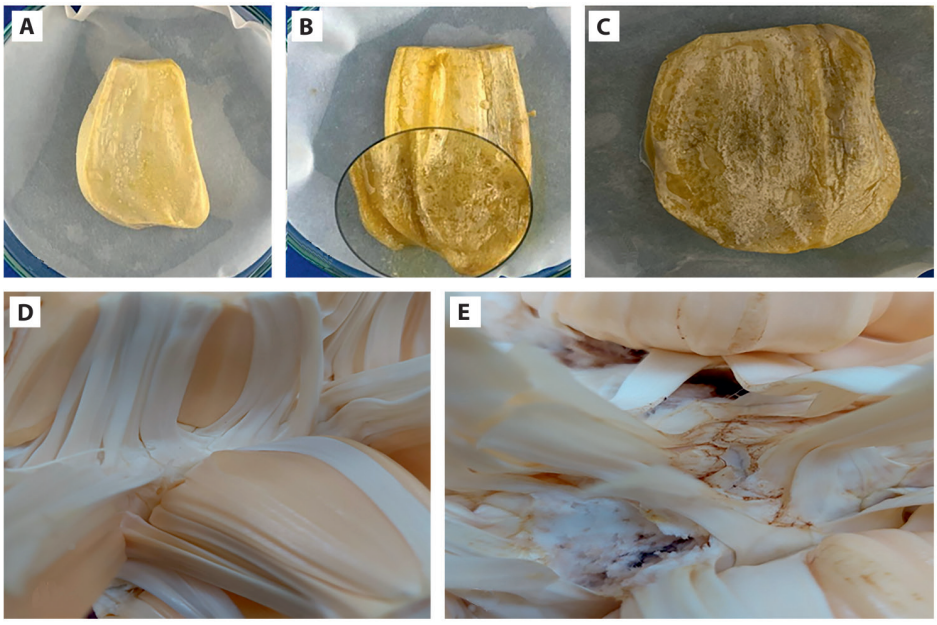

Fig. 2. Bronzing symptoms produced on detached jackfruits 2 weeks after inoculation – B, C, E; control fruit was asymptomatic – A, D

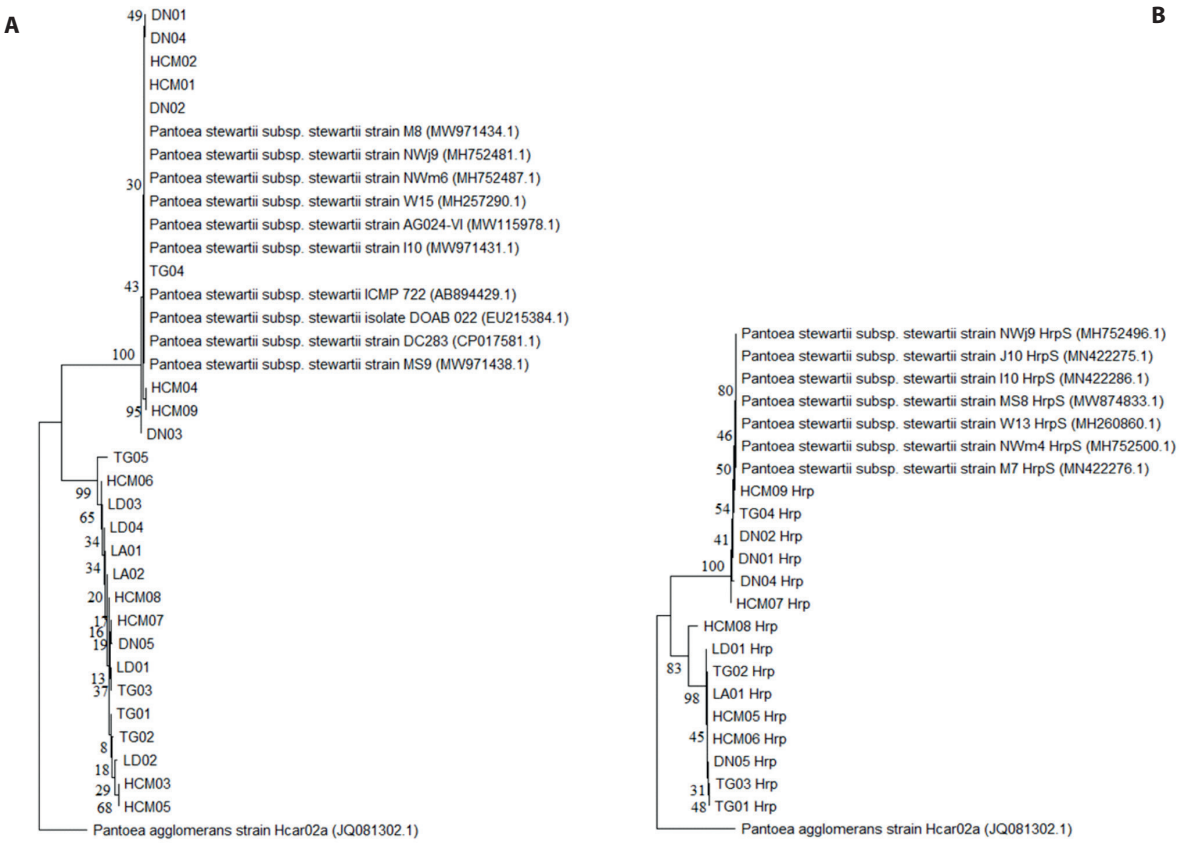

Fig. 3. Phylogenetic tree of bacterial isolates based on sequences: A – *cpsD* gene and B – *HrpS* gene primers with some strains in Gen--Bank. Bootstrap values of 1000 were presented as percentages

The sequences of 14 bacterial strains had a similarity from 98.7 to 99.84% and coverage from 98 to 99% with *P. stewartii* subsp. *stewartii* strain M7 (accession MN422276.1) and strain NWm4 (accession MH752500.1). The phylogenetic tree was constructed using sequences of bacteria *Pss* strain NWj9 (accession MH752496.1), strain J10 *HrpS* (accession MN422275.1), strain I10 *HrpS* (accession MN422286.1), strain MS8 (accession MW874833.1), strain W13 (accession MH260860.1), strain NWm4 (accession MH752500.1) and strain M7 (accession MN422276.1) that a causative agent of bronzing disease on jackfruit in Malaysia, and *P. agglomerans* strain Hcar02a (accession JQ081302.1), was considered as an outgroup, with bootstrap values 1000 time. The branches' bootstrap rate was 31% to 100% (Fig. 3B). Thus, based on the morphological, biochemical, and sequenced *cpsD* and *HrpS* gene results, the causative agents were confirmed to be *P. stewartii* subsp. *stewartii*.

Host range testing on other jackfruit varieties and crops

Cv. Tonu, cv. La Bang, and cv. Nghe varieties are popular traditional jackfruits grown in most southern areas of Vietnam. None of the negative controls of the three jackfruit varieties (cv. Tonu, cv. Nghe, cv. La Bang) injected with water showed any bronzing symptoms. When injected with *Pss* strain HCM09, the pulbof the cv. Tonu variety changed color to dark copper and rotted (Fig. 4E, F) in compared with control (Fig. 4D). No black spots appeared, as described by Abidin (Abidin *et al.* 2021), while the pulbof cv. Labang (Fig. 4C) and cv. Nghe (Fig. 4H, I) varieties rotted, changed color to red copper, and vividly appeared as black spots that were as clear as the positive control sample (Thai variety) (Fig. 4A), while there were no symptoms in control of these varieties (Fig. 4G and Fig. 4B)

The leaves of selected plants for inoculation experiments were free of visible damage or stress. Using consistent leaf age and size to accurately compare experimental groups is essential. When wounding, the *Pss* strain HCM09 generated symptoms on pumpkin and rice leaves as early as 1 day after injection. The disease incidence on pumpkin and rice leaves 7 days after the infection was 100% and 77.7%, respectively. The remaining plants showed symptoms occurring 2 to 3 days after infection; the disease incidence varied between 44.4% and 100% except for guava. No symptoms were observed on guava leaves.

When injecting unwounded leaves, the *Pss* strain HCM09 also exhibited the earliest symptoms for pumpkin and rice on day 2, and for cucumber on the

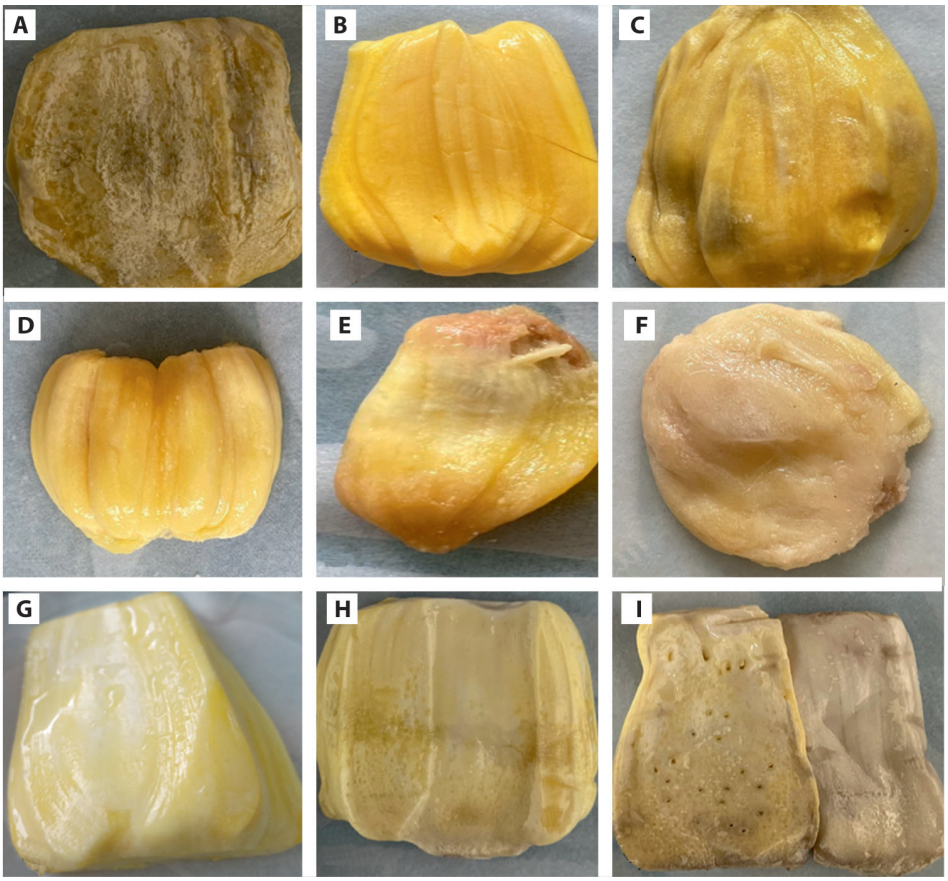

Fig. 4. Symptoms of *Pss* strain HCM09 on three jackfruit varieties. A – control Thai, B – cv. Nghe control, C – cv. Nghe – *Pss* HCM09, D – cv. Tonu control, E, F – cv. Tonu – *Pss* HCM09, G – cv. La Bang control, H, I – cv. La Bang – *Pss* HCM09

fourth day after injection. Disease incidence rose to 77.7%, 22.2%, and 88.8%, respectively, at 7 days following injection. The disease symptoms appeared on green beans and mustard 5 days after injection, with incidence increasing to 100% and 77.7%. The symptoms were not observed on the leaves of the remaining plants.

The experiment showed that damage to the plant tissue was an essential condition for *Pss* strain HCM09 to penetrate and cause disease on various fruits and crops, including jackfruit, mango, longan, durian, tomato, corn, sweet potato, water spinach, and peanuts. Furthermore, without mechanical injuries, *Pss* strain HCM09 could infect other crops like pumpkin, rice, cucumber, green beans, and mustard.

The greenhouse experiment showed that the disease symptoms were observed in all crops when spraying the suspension of the *Pss* strain HCM09 onto two true leaf plants of 10 crops involving tomato, corn, sweet potato, water spinach, peanut, pumpkin, rice, cucumber, green beans, and mustard. The appearance of disease symptoms varied from 1 to 4 days. It was the earliest on rice, corn, water spinach, and the latest on mustard and pumpkin, with the incidence from 33.3 to 60.4%.

Symptoms of the disease caused by *Pss* strain HCM09 varied from crop to crop. The specific symptoms observed depended on the plant's susceptibility, and the standard features were leaf spots or lesions, burns along the leaf margin, and overall decline in plant health (Fig. 5). This suggests that the bacterium *Pss* entered into crop tissue via stomata. The leaf margin was where droplets of moisture containing the bacterial suspensions were retained after spraying (Fig. 5).

Based on these results, the *Pss* strain HCM09 could cross-infect other investigated crops, except for guava. The *Pss* bacterium enters the host plant cell through open wounds or natural openings such as stomata. Rice was the most susceptible crop to *Pss* strain HCM09. This suggests that rice is highly vulnerable to *Pss* and may experience more severe symptoms than other crops when infected with this bacterium. The jackfruit tree should be isolated from durian, longan, and mango trees and not intercropped with tomatoes, broccoli, pumpkin, cucumber, corn, rice, sweet potatoes, water spinach, peanuts, and green beans in the jackfruit garden to prevent cross-contamination and spread of pathogens caused by *Pss.*

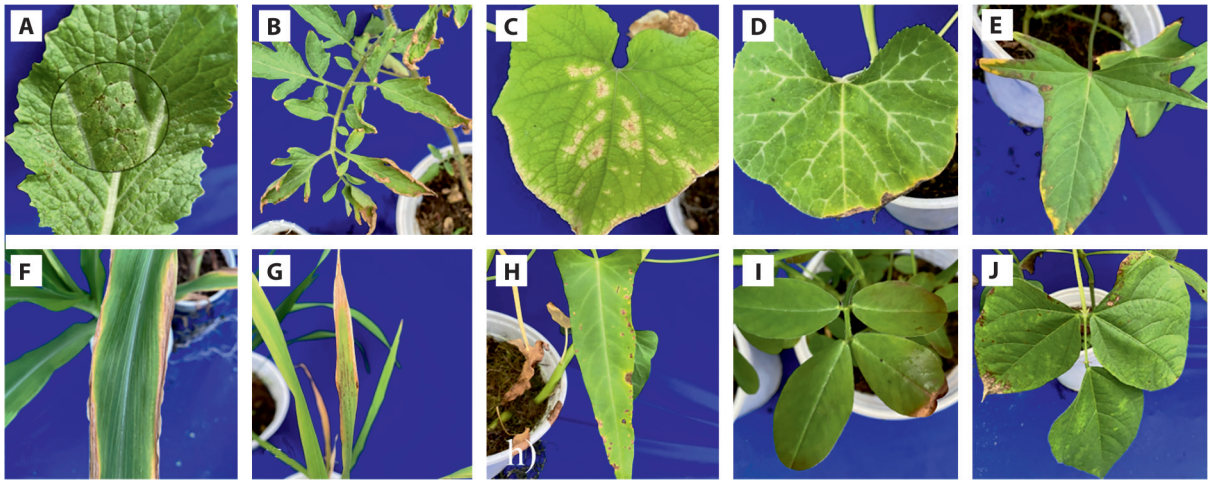

Fig. 5. Symptoms caused by *Pss* strain HCM09 on crops in the greenhouse experiment. A – *Brassica juncea*, B – *Solanum lycopersicum*, C – *Cucumis sativus*, D – *Cucurbita maxima*, E – *Ipomoea batatas*, F – *Zea mays*, G – *Oryza sativa*, H – *Ipomoea aquatica*, I – *Arachis hypogaea* and J – *Phaseolus aureus*

Discussion

The bronzing disease of Thai jackfruit has spread over all cultivated areas in Vietnam. The disease can be identified by the external symptoms of the fruit, such as abnormalities in the fruit stalk, fruit color, fruit shape, and spines, or by being externally symptomless (Vo 2023a). However, disease control is primarily based on a farmer's experience, so the economic losses were high and impossible to measure. This study first identified the causative agent of bronzing of jackfruit from a wide range of regions in southern Vietnam. Based on morphological and biochemical traits, as well as two molecular markers the causative agent of bronzing disease of jackfruit in Vietnam was identified as *P. stewartii*.

For molecular identification, two specific genetic markers were used. The first specific primer pair that targets the *cpsD*/*cpsE* genes in *Pss* is designed to amplify a specific region of the *cpsD* and *cpsE* gene that are involved in the production of exopolysaccharides (EPS), which are essential for biofilm formation and virulence in *P. stewartia* subsp. *stewartii*. The second primer pair that targets the *HrpS* gene in *Pss* is designed to amplify a specific region that encodes regulatory genes essential for the type III secretion system, a key virulence factor in *Pss* (Gapasin *et al.* 2014; Ibrahim *et al.* 2020). These primer pairs were effectively used and support the confirmation of the causative agent of bronzing of jackfruit in Vietnam as described in this study. Although the diseased samples were collected from different provinces and showed missing gaps in sequences of both genes between bacterial strains, they offer high similarities with the GenBank strain. The results of this study confirm the presence of *P. stewartii* subsp. *stewartii* in Vietnam and require appropriate, more careful research on its management.

Most jackfruit plantations in Vietnam are from cultivated rice soil, and almost 20% of Thai jackfruit is planted with traditional jackfruit varieties, other fruits, and crops. Other popular fruits were coconut, pineapple, pomelo, guava, and longan (Vo 2023a), as well as near durian and mango orchards, so testing for possible cross-infection was required. In addition, *Pss* caused severe losses in maize (Scala *et al.* 2023), and could infect cucumber, corn, and pineapple (Abidin *et al.* 2021). This study used the common *Pss* strain, HCM09, for pathogenicity tests with jackfruit varieties, fruits, and crops *in vitro* and under greenhouse conditions.

The *Pss* could affect traditional jackfruit varieties and many fruits and crops under experimental conditions. The *Pss* bacterium can enter the host plant cell through open wounds or natural openings such as stomata. Rice was the most susceptible crop, with or without mechanical injuries. This suggests that rice is highly vulnerable to *Pss* and may experience more severe symptoms than other crops when infected with this bacterium. It is recommended that the Thai jackfruit tree should be isolated from durian, longan, and mango trees and not intercropped with tomatoes, broccoli, pumpkin, cucumber, corn, rice, sweet potatoes, water spinach, peanuts, and green beans in the jackfruit plantations to prevent cross-contamination and spread of pathogens caused by *P. stewartii* subsp. *stewartii*.

Conclusions

Bacteria *Pantoea stewartia* subsp. *stewartii,* the causative agent of jackfruit's bronzing, was identified based on morphological and molecular characteristics. The bacterium produces white or yellow pigment in King'B medium, is gram-negative, slightly pleomorphic, nonmotile, facultatively anaerobic, short-rod, and catalase-positive. It hydrolyzes gelatin and starch but not tween 80, and produces acid from glucose, sucrose, and lactose. The bacterium does not produce indole and does not produce hypersensitivity to tobacco. The results of sequencing of the encoded region of synthesis of capsular polysaccharide (*cpsD*) and pathogenicity-related genes *HrpS* confirmed that the causative agent was *P. stewartii subsp. stewarti.* The host range of *Pss* strain HCM09 could be traditional jackfruit, fruits like durian, longan, and mango, as well as crops like tomatoes, broccoli, pumpkin, cucumber, corn, rice, sweet potatoes, water spinach, peanuts, and green beans.

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