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Erwinia mallotivora is the causal agent of papaya bacterial crown rot disease in Lampung Timur, Indonesia

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Abstract: Sixteen bacterial strains showing oval, convex with a white colony colour were obtained from the water-soaked lesions on the petioles and leaves of infected papaya (cv. *calina*) collected from a papaya field in Lampung Timur, Indonesia. The pathogenicity test showed that all the strains produced the same symptoms with those found in the field. Four representative strains were then chosen for further investigation. The phenotypic characteristics revealed that the strains resembled *Erwinia mallotivora*. Two representative strains were further identified using a 16SrDNA sequence analysis. The result showed that the strains were placed within the group of the type strain and the reference strains of *E. mallotivora*. To the best of our knowledge, this is the first finding of *E. mallotivora* as the causal agent of papaya crown rot disease in Indonesia. Among the sixteen plants used for the host range test, the symptom was only observed on eggplants, but not on the other fifteen plant species.

Keywords: eggplant; host range test; identification; sequence analysis of 16SrDNA; water-soaked lesions

An outbreak of papaya bacterial disease was observed in October 2017 at Lampung Timur, Indonesia. It was initially recognised by the water-soaking lesions on the basal petioles and it would expand to the crown. Further progress of infection caused the crown rot disease and the plant eventually died (Figure 1A). Sometimes, the symptoms were also established on the leaves' surface (Figure 1B). Since its discovery, the disease has then also been found in the other papaya production area in Lampung causing severe damage up to 100% yield loss, one of which was in Tanggamus (Figure 1C).

The crown rot disease of papaya in Indonesia was firstly reported by von Rant (1931) at Java Island and the pathogen was named *Bacillus papayae*. The bacteria were then placed within genus *Erwinia* by Magrou (1937). Later, Gardan et al. (2004) proposed a new name of the pathogen as *Erwinia papayae*. This pathogen has been reported causing papaya bacterial canker in the Caribbean islands (Gardan et al. 2004). In this study; however, we found that in the advanced stage, the bacteria causing papaya crown rot in Lampung Timur did not cause any canker symptoms indicating that the pathogen might be different from those reported by Gardan et al. (2004).

A similar disease on papaya with no canker symptoms was also reported by Maktar et al. (2008) in Malaysia and it was identified as *E. papayae*. However, it was later confirmed by Amin et al. (2011)

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(A)

(B)

(C)

Figure 1. (A) Crown rot symptom observed water-soaked symptom on basal petioles which had already spread to the crown, (B) symptom observed on the leaf surface and (C) damage caused by the disease, causing the death of all papaya plants in one papaya field at Tanggamus

that the causal agent of the papaya disease reported by Maktar et al. (2008) was not *E. papayae*, but *Erwinia mallotivora*.

This study aimed at characterising and revealing the identity of the causal agent of the bacterial crown rot disease of papaya in Lampung Timur based on the phenotypic characteristics and sequence analysis of the 16SrDNA as well as to identify its host range other than the papaya. Elucidating the identity and host range of the causal agent is the first step to find an effective controlling method in order to prevent further economic losses due to the enormous damage caused by the pathogen.

MATERIAL AND METHODS

Isolation of the pathogen

Isolation was performed on the water-soaked lesions on the petioles and leaves collected from naturally infected papaya (cv. *calina*) in the field at Lampung Timur, Indonesia in October 2017. The margin between the diseased and healthy area of the symptomatic petioles and leaves were cut into pieces of 0.5 × 0.5 cm and surface sterilised by dipping them into 70% ethanol for 3 seconds and then directly putting them into a 1.5 mL tube containing 500 µL of sterile distilled water, then macerated and allowed to stand for 10 minutes. The tissue extract was then streaked onto a nutrient agar medium (Oxoid, England) and incubated at room temperature for 48 hours. A single colony of the suspected pathogens was collected and transferred into a potato peptone glucose agar (PPGA) media (200 g of potato, 5 g of peptone, 5 g of glucose, 3 g of $Na_2HPO_4 \cdot 2H_2O$, 3 g of NaCl, 0.5 g of KH_2PO_4, 20 g of agar, 1 000 mL of distilled water) (Nishiyama 1978) slant for further investigation. The suspected pathogens were chosen on the colonies showing a convex, oval, shiny, creamy-white colour with slow growth. The confirmed pathogens were then preserved in a skim milk media (5 g of skim milk, 0.75 g of Na glutamate, 50 mL of distilled water) (Suharjo et al. 2014) and stored at –40 °C.

Pathogenicity test

A three-month-old healthy papaya (cv. *calina*) was used for the pathogenicity test. The inoculation was onducted by stabbing a drop of the bacterial suspension (~ 10^8 CFU/mL) in the basal petiole and adaxial leaves' surface using a 1 mL sterile syringe. The inoculation sites were observed every day for 21 days, where some water-soaked lesions emerged.

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Phenotypic characterisation

The gram reaction test was performed with the non-staining method using 3% KOH (Ryu 1940). The production of a fluorescent pigment was observed on King's B medium (King et al. 1954). The Oxidation and Fermentation (OF) test was conducted using the OF medium described by Hugh and Leifson (1953). The potato soft rot and lecithinase tests were conducted based on the method described by Lelliot et al. (1966). The Arginine Dihydrolase (ADH) Moeller test was performed using a Moeller Decarboxylase Broth (Himedia, India) medium based on the method described by Dickey (1979). The growth capability at a 5% concentration of NaCl was performed based on the methods described by Dye (1968). The gas production from H_2S, reducing the substance from the sucrose, and hypersensitive reaction test on the tobacco leaves were conducted based on Schaad et al. (2001). The purple pigment on the PPGA medium was performed by streaking the 24-hour old bacterial strain on the PPGA slant medium described by Nishiyama (1978). The utilisation of seventeen organic compounds as a source of carbon was conducted using the modified Ayers Medium (Society of American Bacteriologist 1957) that was incorporated with 0.1% (w/v) of organic compounds. A positive reaction was recorded when the bacteria grew on the medium that was incubated at 27 °C. Observations took place at 2, 4, 7 and 21 days after the inoculation. The growth capability at 36, 37, 39, 40 and 41 °C was conducted using a yeast peptone (YP) broth medium (5 g of yeast extract, 10 g of peptone in 1 000 mL of distilled water) (Suharjo et al. 2014).

Molecular identification.

DNA extraction. The DNA extraction was performed from the 24-hour-old bacteria cultured on the PPGA. A loop of bacteria was directly taken from the PPGA and put into a sterile 1.5 mL tube containing 20 µL of DNAzol™ (ThermoFisher, USA) and its DNA was extracted following the company's recommendations.

PCR amplification. Polymerase chain reaction (PCR) test was performed using a SensoQuest (SensoQuest GmbH, Germany) thermal cycler machine. The amplification was conducted in a total volume of 25 µL containing 1 µL of the DNA template (~ 1 µg/µL of concentration), 12.5 µL of MyTaq™ Red Mix (Bioline, USA) and 1 µL of primer fD1 (CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG) and rP2 (CCCGGGATCCAAGCTTACGGCTACCTTGTTACGACTT) (10 µM in concentration) which amplified the 16SrDNA region with 1 500 bp of the PCR product (Weisburg et al. 1991). The PCR amplification was achieved with 1 cycle of an initial denaturation at 94 °C for 5 min, 30 cycles contained of denaturation at 94 °C for 1 min, primer annealing at 58 °C for 1 min, a primer extension at 72 °C for 1 min, and 1 cycle of a final extension at 72 °C for 5 min. The PCR product was electrophoresed in a 0.5% agarose gel dissolved with a Tris-Boric Acid-EDTA (TBE) buffer (pH 8.0) (Invitrogen, USA) containing ethidium bromide (10 mg/mL) which was deep within the TBE buffer (pH 8.0) (Invitrogen, USA) at 50 V for 70 min. The result was visualised under a DigiDoc UV trans-illuminator (UVP, USA). The PCR products were sent to 1st Base, Malaysia for sequencing.

Phylogenetic analysis. The 16SrDNA sequence was analysed using the BioEdit for Windows program (version 7.2.6) (Hall 1999). The phylogenetic tree was developed with a neighbour-joining method (Jukes and Cantor model) using MEGA7 (version 7.0) for Windows (Kumar et al. 2016). The 16SrDNA sequence of the *Erwinia* species reference strains were retrieved from the NCBI GenBank (https://www.ncbi.nlm.nih.gov/). The accession number of the retrieved sequence can be seen in Table 1.

Host range test. The host range test was performed on sixteen kinds of plants species other than papaya (aloe vera, celery, Welsh onion, tomato, choi sum, Chinese cabbage, cabbage, bean, eggplant, chayote, onion, carrot, chili, cucumber, lettuce and pak choi) using the same method as described in the pathogenicity test. The inoculated plant parts were transferred into plastic trays (40 × 40 × 60 cm) covered by cling wrap and incubated at room temperature (Aeny et al. 2020). Observation on the necrotic or soft rot symptoms, which emerged on the inoculated area, was performed every day for 7 days.

RESULTS

Bacterial isolates. Sixteen bacterial colonies determined as the causal agent were isolated from the symptoms on the petioles and leaves of the papaya. On the nutrient agar (NA) medium, the colonies were oval, convex with a creamy-white colony colour; however, at 72 h after inoculation, the colonies' colour turned purple (Figure 2).

Pathogenicity test. All sixteen bacterial strains obtained produced the same symptoms as found

https://doi.org/10.17221/123/2020-PPS

Table 1. Reference strains used in this study

Species	Strain	Other designation	Accession No.	Reference
E. amylovora	ATCC15580.T	CIP82.82, DSM30165, LMG2024, NCAIMB.01108, NCPPB683, PDDCC1540	U80195	Kwon et al. 1997
E. aphidicola	GTC1688.T	IAM14479	AB273744	Nhung et al. 2007
E. bilingiae	LMG2613.T	DSM17872, NCPPB661	NR118431	Unpublished
E. endophytica	BSTT30.T	LMG28457, CECT8692	NR148650	Ramirez-Bahena et al. 2016
E. gerundensis	EM595.T	LMG28990, CCOS903	NR148820	Rezzonico et al. 2016
E. iniecta	B120.T	CFBP8182, NCCB100485	KM870781	Campillo et al. 2015
E. mallotivora	BT-MARDI	–	HQ456230	Amin et al. 2011
	DSM4565.T	ATCC29573, NCPPB2851, PDDCC5705	AJ233414	Sproer et al. 1999
E. oleae	DAPP-PG531.T	DSM23398, KCTC42565, LMG25322	GU810925	Moretti et al. 2011
E. papayae	ICMP14628	–	MF682394	Unpublished
	CFBP11606	–	NR042748	Gardan et al. 2004
	CFBP5189.T.	DSM16540, NCPPB4294	AY131237	
E. persicina	ATCC35998.T	DSM19328, IAM12843, JCM3704	U80205	Kwon et al. 1997
E. piriflorinigrans	CECT7348.T	DSM26166, CFBP5888	GQ405202	Lopez et al. 2011
E. psidii	LMG7039.T	DSM17597, LMG7039, NCPPB3555, IBSBF435, PDDCC8426	JQ809696	Unpublished
E. pyrifoliae	CFBP4172.T	CIP106111, DSM12163	AJ009930	Kim et al. 1999
E. raphontici	DSM4484.T	ATCC29283, NCPPB1578	AJ233417	Sproer et al. 1999
E. tasmaniensis	Et1/99.T	DSM17950, CIP109463	NR074869	Kube et al. 2008
E. teleogrylli	SCU-B244.T	DSM28222, CGMCC1.12772, KCTC 42022	KF500917	Liu et al. 2016
E. toletana	A37.T	DSM18073, ATCC700880, CECT5263, CFBP6631	AF130910	Rojas et al. 2004
E. tracheiphila	LMG2906.T	DSM21139, CFPB2355, CIP105205, NCPPB2452	Y13250	Hauben et al. 1998
E. typographi	Y1.T	DSM22678, LMG25347	GU166291	Skrodenyte-Arbaciauskiene et al. 2012
E. uzenensis	YPPS951.T	LMG25843, NCPPB4475	AB546198	Matsuura et al. 2012
Pseudmonas aeruginosa	LMG1241.T	ATCC19194, CIP70.27	Z76651	Moore et al. 1997

ATCC – American Type Culture Collection, Manassas, USA; CCOS – Culture Collection of Switzerland, Zurich University of Applied Sciences, Switzerland; CCM – Czech Collection of Microorganisms, Masaryk University, Czech Republic; CECT – Colección Española de Cultivos Tipo, University of Valencia, Spain; GTC – Gifu Type Culture Collection, Gifu 501-1194, Japan; CFBP – Collection FranCaise des Bactiries Phytopathogenes INRA, France; CGMCC – China General Microbiological Culture Collection Center, Chinese Academy of Sciences China; CIP – Collection de L'Institut Pasteur institut Pasteur, France; DSM – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany; IAM – Institute of Applied Microbiology, University of Tokyo, Japan; IBSBF – Phytobacteria Culture Collection of Instituto Biológico Instituto Biologico, Sâo Paulo, Brazil; ICMP – International Collection of Microorganisms from Plants, Auckland, New Zealand; JCM – Japan Collection of Microorganisms, RIKEN BioResource Research Center, Ibaraki, Japan; KCTC – Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, Korea; LMG (BCCM/LMG) – Belgian Coordinated Collections of Microorganisms/ LMG Bacteria Collection, Universiteit Gent - Laboratorium Voor Microbiologie, Belgium; NCAIM – National Collection of Agricultural and Industrial Microorganisms, Szent Istvan University, Hungary; NCPPB – National Collection of Plant Pathogenic Bacteria, Fera Science Ltd. UK; PDDCC – Culture Collection of the Plant Disease Division, Department of Scientific and Industrial Research, Auckland, New Zealand; T – type strain

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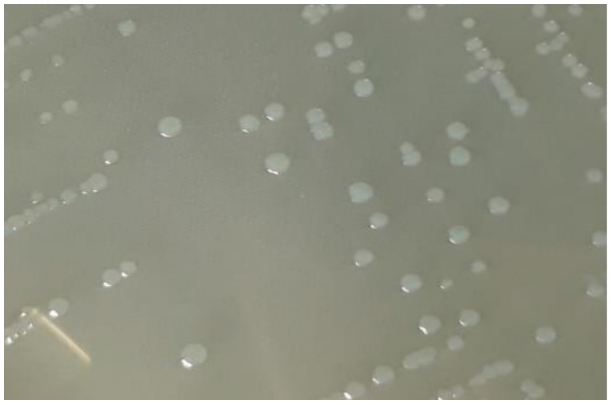

Figure 2. Bacterial colonies obtained in this study which were cultivated on an NA medium 72 hours after inoculation

in the field (Figure 3). The water-soaked spots were initially observed at 3 days after inoculation followed by crown rot at 12 days after inoculation and the plant was completely dead at 21 days after inoculation. Four representative strains (PPY1TDU2, PPY2DU2, PPY2TDU3 and PPY2TDU4) were chosen for further investigation.

Phenotypic characteristics. The results of the phenotypic tests are shown in Table 2. All of the bacterial isolates showed the same phenotypic characteristics. The bacteria were gram negative bacteria, fermentative, had a positive hypersensitive reaction on the tobacco leaf (Figure 4A), produced a purple pigment on the yeast extract-dextrose-CaCO3 composition (YDC) (Figure 4B) and PPGA medium (Figure 4C), were positive on the ADH Moeller test, were positive on the reducing substance from the sucrose test, showed at rowth capability at 36–40 °C and were able to utilise D-mannitol, Myo-inositol, L-tartrate, glycerol, cis-aconitic acid and citrate as their carbon source. They showed a negative result on lecithinase test, did not produce fluorescent pigment on King's B medium, did not produce gas from H_2S, could not grow in 5% NaCl and were unable to utilise M-tartrate, D-raffinose, D-arabinose, D-melibiose, inulin, D-tartrate, starch, 5-ketogluconate, lactose, L-ascorbic acid, and ascorbic acid.

Molecular identification. Two bacterial strains (PPY2TDU3 and PPY2DU2) were chosen as the representative strains used in this study for the molecular identification. The BLAST search result showed that the 16SrDNA sequence of the respective strains shared a 99.85% sequence similarity with *E. mallotivora* BT MARDI (Acc. No. HQ456230) and a 99.20% sequence similarity with the DSM 4565 type strain of *E. mallotivora* (Acc. No. AJ233414). Both respective pathogens also showed a 99.20% sequence similarity with *E. papayae* ICMP14628 (Acc. No. MF682394). The nucleotide difference showed that PPY2TDU3 and PPY2DU2 are closer to the type strain and reference strains of *E. mallotivora* than to that of the type strain and reference strains of *E. papayae* (Table 3). The result of the phylogenetic tree analysis showed that the strains were placed in the group of type strain (DSM4565.T Acc no. AJ233414) and reference strain of *E. mallotivora* (BT-MARDI, Acc no. HQ456230) (Figure 5).

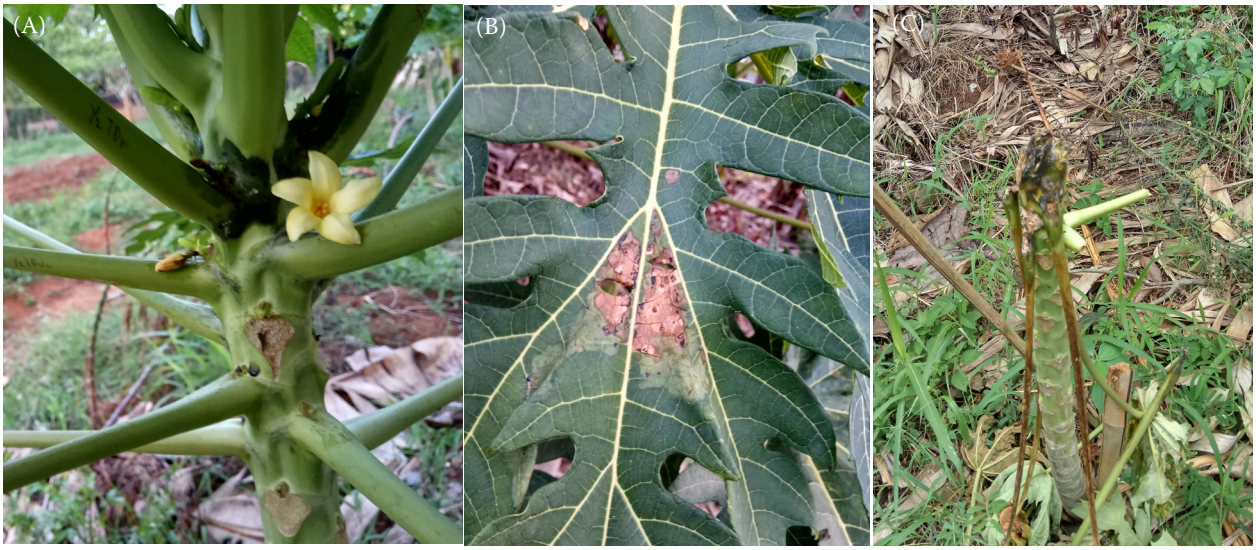

Figure 3. Results of the pathogenicity test on papaya (cv. *calina*); (A) symptoms on the petiole (B) the leaves and (C) the dead papaya plant

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Table 2. Phenotypic characteristics of the bacterial strains

Phenotypic test	PPY1TDU2	PPY2DU2	PPY2TDU3	PPY2TDU4
Gram reaction	–	–	–	–
OF test	F	F	F	F
Lecithinase	–	–	–	–
Potato soft rot	–	–	–	–
Hypersensitive on tobacco leaves	+	+	+	+
Blue pigment on King's B medium	–	–	–	–
Blue pigmen on YDC agar medium	+	+	+	+
Gas production from H_2S	–	–	–	–
Growth in 5% NaCl	–	–	–	–
Arginine dihydrolase Moeller	+	+	+	+
Blue pigmen on PPGA medium	+	+	+	+
Growth capability at				
36 °C	+	+	+	+
37 °C	+	+	+	+
39 °C	+	+	+	+
40 °C	+	+	+	+
Reducing substance from sucrose	+	+	+	+
Utilisation of				
Myo-inositol	+	+	+	+
M-tartrate	–	–	–	–
D-raffinose	–	–	–	–
D-arabinose	–	–	–	–
D-melibiose	–	–	–	–
Inulin	–	–	–	–
L-tartrate	+	+	+	+
D-tartrate	–	–	–	–
D-mannitol	+	+	+	+
Glycerol	+	+	+	+
Starch	–	–	–	–
S-ketogluconate	–	–	–	–
Lactose	–	–	–	–
Cis-aconitic acid	+	+	+	+
L-ascorbic acid	–	–	–	–
Ascorbic acid	–	–	–	–
Citrate	+	+	+	+

OF test – oxidation and fermentation test; F – fermentative; YDC – yeast extract-dextrose-CaCO3; PPGA – potato peptone glucose agar

Host range test. All the strains showed the capability to infect and produce rotting symptoms on the eggplant, but not on the celery, carrot, aloe vera, curly lettuce, Chinese cabbage, cabbage, choi sum, pak choi, chayote, cucumber, green bean, onion, Welsh onion, chili and tomato (Table 4). The symptoms appeared at 24 hours after inoculation (Figure 6).

DISCUSSION

Four bacterial strains, as representative strains, selected from the sixteen bacterial strains obtained from the water-soaking symptoms on the petioles and leaves of the papaya, were further investigated on their characteristics and identity as well as their capability to infect and cause symptoms to host

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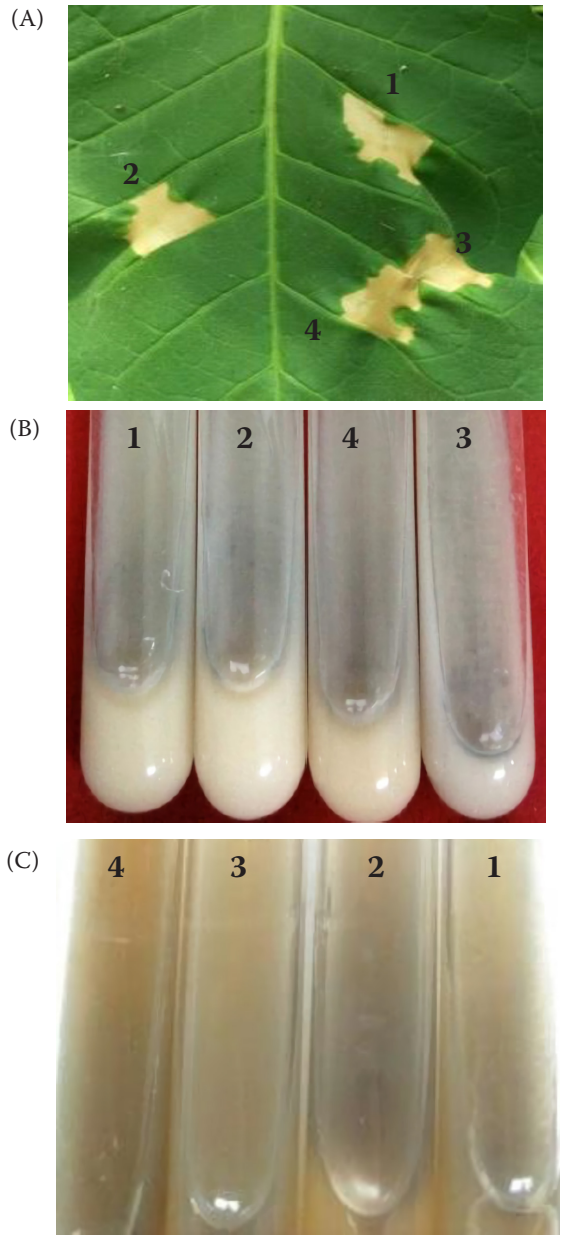

Figure 4. (A) Hypersensitive test on tobacco leaves and purple pigment production on the (B) YDC and (C) PPGA medium

1 – PPY1TDU2; 2 – PPY2DU2; 3 – PPY2TDU3; 4 – PPY-2TDU4; YDC – yeast extract-dextrose-CaCO3; PPGA – potato peptone glucose agar

plants other than papaya. Identification was performed based on the phenotypic characteristics and the 16SrDNA sequence analysis. The host range test was conducted on eight plant families consisting of sixteen plant species (Table 4).

At advanced stages of infection, crown rot was observed leading to its causal agents as *E. papayae* (vont Rant 1931; Gardan et al. 2004; Semangun

Table 3. Position of nucleotide difference among *Erwinia mallotivora* and *Erwinia papayae*

Name of the strains	Position of nucleotide difference																					
	5	100	101	102	103	163	368	369	373	374	384	386	389	390	475	923	936	939	976	1047	1053	1194
PPY2DU2*	C	G	C	A	A	G	T	G	A	G	C	T	C	A	T	T	G	A	C	G	C	C
PPY2TDU3*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. mallotivora BT MARDI	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	.	.	.	.
E. mallotivora DSM4565.T	T	T	T	C	G	.	.	.	G	.	.	.	.	.	.	.	T	G	.	A	T	T
E. papayae ICMP14628	.	.	.	.	.	A	G	A	G	A	T	C	T	C	.	.	T	G	.	.	.	.
E. papayae CFBP11606	.	.	.	.	.	A	G	A	G	A	T	C	T	C	G	C	T	G	G	.	.	.
E. papayae CFBP5189.T	.	.	.	.	.	A	G	A	G	A	T	C	T	C	G	C	T	G	G	.	.	.

*strains used in this study; T– type strain

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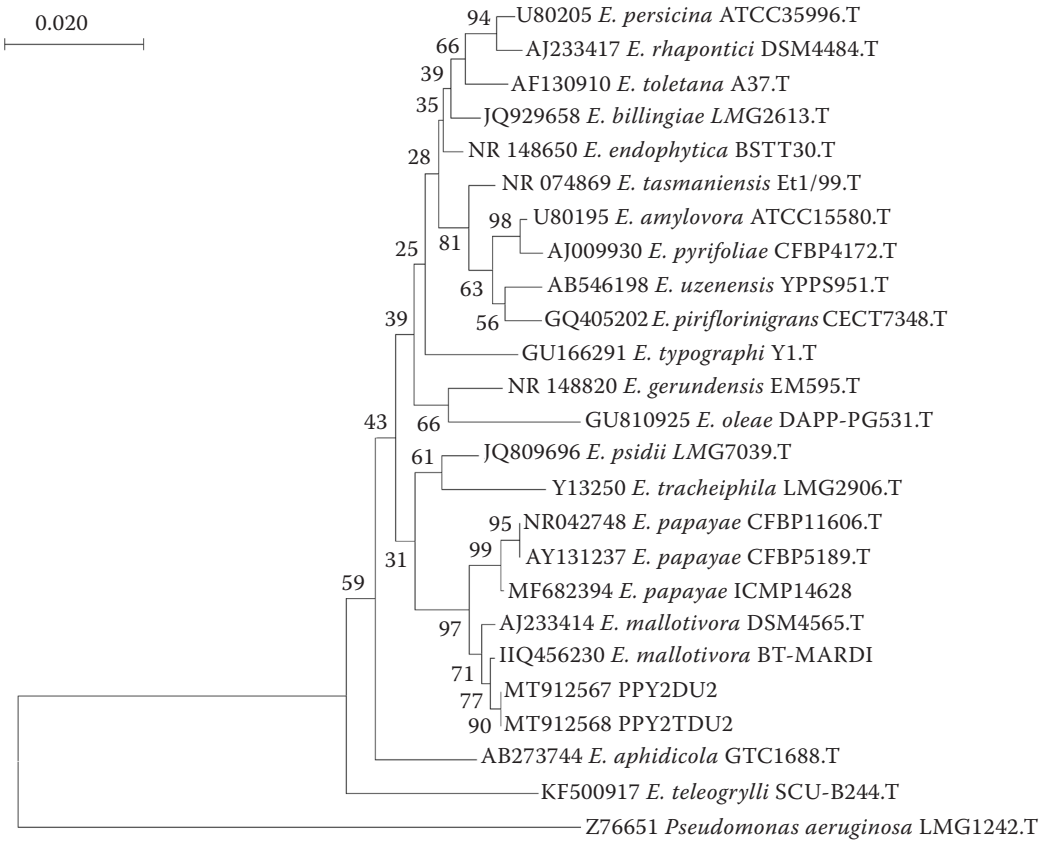

Figure 5. Phylogenetic tree developed based on the 16SrDNA sequence analysis using the neighbour-joining method (Jukes and Cantor model) created using MEGA7 for windows

The strains used in this study (PPYDU2 and PPYTDU3) were placed within the type strain (T) and reference strains of *E. mallotivora*; the strains have been deposited into the GenBank with Acc. No MT912567 (PPYDU2) and MT912568 (PPYTDU3); the type strain of *Pseudomonas aeruginosa* LMG1242.T. (Acc. No. Z76651) was used as the outgroup

Table 4. Plants used for the pathogenicity test

Family	Species name	Common name	Inoculated part	Strains			
				PPY1TDU2	PPY2DU2	PPY2TDU3	PPY2TDU4
Apiaceae	*Apium graveolens*	celery	stem	–	–	–	–
	Daucus carota	carrot	root	–	–	–	–
Asphodelaceae	*Aloe vera*	aloe vera	leaf	–	–	–	–
Asteraceae	*Lactuca sativa*	curly lettuce	leaf	–	–	–	–
Brassicaceae	*Brassica chinensis*	Chinese cabbage	leaf	–	–	–	–
	Brassica oleracea	cabbage	leaf	–	–	–	–
	Brassica rapa	choi sum	leaf	–	–	–	–
	Brassica rapa subsp. *chinensis*	pak choi	leaf	–	–	–	–
Cucurbitaceae	*Sechium edule*	chayote	fruit	–	–	–	–
	Cucumis sativus	cucumber	fruit	–	–	–	–
Fabaceae	*Phaseolus vulgaris*	green bean	pod	–	–	–	–
Liliaceae	*Allium cepa*	onion	bulb	–	–	–	–
	Allium fistulosum	Welsh onion	leaf	–	–	–	–
Solanaceae	*Capsicum annuum*	chili	fruit	–	–	–	–
	Solanum lycopersicum	tomato	fruit	–	–	–	–
	Solanum melongena	eggplant	fruit	+	+	+	+

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Figure 6. The result of host range test (A) carrot, (B) celery, (C) aloe vera, (D) curly lettuce, (E) Chinese cabbage, (F) cabbage, (G) choi sum, (H) pak choi, (I) chayote, (J) cucumber, (K) green bean, (L) onion, (M) Welsh onion, (N) chili, (O) tomato and (P) eggplant

A positive result was only observed on the eggplant; circle – inoculated site

2007) or . *mallotivora* (Amin et al. 2011; Cueva et al. 2017). Based on the phenotypic characteristics, *E. mallotivora* and *E. papayae* can be generally differentiated on their capability to utilise citrate, D-mannitol, L-arabinose, reducing the substance from the sucrose, the hypersensitive reaction on the tobacco leaves, and producing a blue pigment on King's B Medium (Goto 1976; Gardan et al. 2004; Amin et al. 2011). The strains used in this study showed the capability to utilise citrate, D-mannitol, but not L-arabinose. They were able to reduce the substance from the sucrose as well as induce a hypersensitive reaction on the tobacco leaves; however, they did not produce a blue pigment on King's B Medium.

https://doi.org/10.17221/123/2020-PPS

These characteristics resemble *E. mallotivora* reported by Amin et al. (2011) and Goto (1976). The advanced observations revealed that the strains showed a purple colony colour at 72 hours after inoculation and it was consistently observed when they were cultivated on the YDC and PPGA slant medium. *E. papayae* have not been reported to produce a purple pigment during their growth (Trujillo & Scroth 1982; Webb 1985; Olabiyi 2010). Nelson and Alvarez (1980) reported bacterial strains showed a purple colony colour causing the purple disease of papaya fruit and it was described as *E. herbicola*. Fullerton et al. (2011) found bacterial colonies with a blue colour causing papaya crown rot disease. However, based on the phenotypic characteristics and sequence analysis of 16SrDNA, there was insufficient evidence to determine the strains as *E. papayae*. Here, Fullerton et al. (2011) named the pathogen as *E. papayae*-like bacterium. Further observations showed that the strains did not produce canker symptoms as those produced by *E. papayae* reported by Gardan et al. (2004) as well as other scientists (Trujillo & Scroth 1982; Webb 1985; Olabiyi 2010). Amin et al. (2011) reported *E. mallotivora*, which was found to be the causal agent of papaya crown rot in Peninsular Malaysia, did not produce any canker symptoms. This finding indicated that the strains were suggested to be *E. mallotivora*.

The sequence analysis result of 16SrDNA showed that the PPY2TDU3 and PPY2DU2 shared a 99.85% sequence similarity with *E. mallotivora* BT MARDI (Acc No. HQ456230) and a 99.20% sequence similarity with the DSM 4565 type strain of *E. mallotivora* (Acc. No. AJ233414). They also shared a 99.20% sequence similarity with *E. papayae* ICMP14628 (Acc. No. MF682394). The high similarity of the 16SrDNA sequence between *E. mallotivora* and *E. papayae* were also reported by Gardan et al. (2004) and Amin et al. (2011). The CFBP5189 type strain of *E. papayae* (Acc. No. AY131237) shared a 98.6% sequence similarity with the DSM4565 type strain of *E. mallotivora* (Acc. No. AJ233414) (Gardan et al. 2004), meanwhile *E. mallotivora* BT-MARDI (HQ456230) shared a 99% sequence similarity with the CFBP5189 type strain of *E. papayae* (Acc. No. AY131237) (Amin et al. 2011). In line with those stated by Gardan et al. (2004) and Amin et al. (2011), this study also revealed that *E. mallotivora* and *E. papayae* are closely related. On the basis of the 16SrDNA nucleotide difference analysis, it showed that PPY2TDU3 and PPY2DU2 were closer to the group of *E. mallotivora* than to that of *E. papayae* (Table 3). The phylogenetic tree analysis revealed that the strains were placed in the group of type strain (DSM 4565.T Acc No. AJ233414) and reference strain (BT-MARDI, Acc No. HQ456230) of *E. mallotivora*. This result confirmed that the identity of the strain was *E. mallotivora*.

E. mallotivora was firstly found in *Mallotus japonicus* causing dark-brown leaf spot and shoot blight (Goto 1976). The pathogen has been recognised as an insignificant disease on *M. japonicus*, which was only a mild symptom affecting the leaves and did not cause death of the shoot (Goto 1976). Since then, there have been no reports on any host plant other than *M. japonicus*. In 2011, *E. mallotivora* was reported as the causal agent of papaya crown rot in Peninsular Malaysia (Amin et al. 2011). Five years later, the pathogen was also reported in the Philippines affecting papaya plants causing the same disease as was previously reported in Malaysia (Cueva et al. 2017). In Indonesia, the crown rot disease of papaya has been described as solely being caused by *E. papayae* (Semangun 2007). This study, however, revealed that *E. mallotivora* has also become the pathogen of papaya crown rot disease in Indonesia which was mostly found in cv. *calina*. In order to reveal the taxonomic relationship between *E. mallotivora* from papaya, *E. mallotivora* from *M. japonicus* and *E. papayae*, a re-investigation using type strains on their phenotypic characteristics as well as a cross inoculation both on papaya and *M. japonicus* should be performed.

Several papaya cultivars have been reported to be susceptible to *E. papayae*, such as cv '*solo*' (Trujillo & Scroth 1982; Webb 1985; Gardan et al. 2004; Cueva et al. 2017), Tainung (Gardan et al. 2004), S-64, PR6-65, PR9-65, PR 7-65, PR 8-65, CATIE, CVI, JH, Trinidad Pink X and Yellow (Webb 1985). Only a few papaya cultivars were reported to be least tolerant to *E. papayae*, namely the Barbados Dwarf 2x, Trinidad Pink, STT 683-1 and PR 10-65 (Webb 1985). So far, the capability of *E. mallotivora* to cause diseases on other cultivars of papaya have not been elucidated upon. In order to obtain comprehensive control strategies, it is strongly recommended to perform pathogenicity tests on other papaya cultivars.

The host range test discovered that the *E. mallotivora* strains used in this study only caused symptoms on the eggplant fruit, not on the other fifteen plant species. It indicated that the pathogen had been restricted to the host plant. In the case of *E. papayae*, the study performed by Webb (1985)

https://doi.org/10.17221/123/2020-PPS

on the survival of *E. papayae* on leaves of eighteen plant species showed that at 7 days after inoculation, the pathogen can only be recovered from the cowpea, melon and tomato. At 14 days after inoculation, an increase in the recovered pathogen was observed merely on the melon and tomato. A further study performed by Olabiyi (2010) revealed that among thirteen plant species inoculated with *E. papayae*, only two species (Siam weed and tomato) showed potential as being an alternative host of the pathogen. The pathogen can be recovered from these two plants 7 days after inoculation, but not from the other tested plants. After 14 days of inoculation, the population of the pathogen was too low to be detected or completely disappeared. In the case of *E. mallotivora*, however, there is no report on the host range other than *M. japonicus* and papaya. The information obtained in this study can be used as a preliminary report to reveal the host range of this pathogen.

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