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Characterization and host range assessment of *Dickeya zeae* associated with pineapple soft rot disease in East Lampung, Indonesia

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[2]Department of Agronomy and Horticulture, Faculty of Agriculture, Universitas Lampung, Jl. Prof. Sumantri Brojonegoro No. 1, Bandar Lampung 35145, Lampung, Indonesia

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Abstract. *Aeny TN, Suharjo R, Ginting C, Hapsoro D, Niswati A. 2020. Characterization and host range assessment of Dickey zeae associated with pineapple soft rot disease in East Lampung, Indonesia. Biodiversitas 21: 587-595.* The study aims to characterize the *Dickeya zeae* associated with pineapple soft rot in East Lampung, Indonesia and to assess the bacterial host range. From the blister-like lesion-symptom, bacteria were isolated with the morphological characteristics: circular, convex, cream white milk-colored, with diameter colonies ranging from 1-2 mm in diameter. Two strains (N-Unila 5 and N-Unila 10) were selected for further investigation including pathogenicity test on pineapple seedling, species identification by phenotypic characteristics and molecular techniques using sequence analysis of 16SrDNA, *recA*, and *dnaX* as well as host range test on 25 different plant species. The result of the pathogenicity test showed similar symptoms to those observed in the field. Physiological and biochemical characterization revealed that the two isolates were Gram-negative bacteria, fermentative, lecithinase positive, non-fluorescent on King's B medium, able to grow on YDC medium at 41°C, did not produce H_2S and did not grow in the presence of 5% NaCl. The isolates capable of using Myo-inositol, M-tartrate, mannitol, L-tartrate, lactose, glycerol, D-melibiose, D-arabinose, citrate, and cis-aconitid but did not utilize starch, S-ketoglucanate, L-ascorbic acid, inulin, folic acid, D-raffinose and tartrate as a sole carbon source. Phenotypic characteristics indicated that the strains were in the group of *Dickeya* spp. bv. 3 (phenon 1). Sequence analysis of 16S rDNA, *recA,* and *dnaX* revealed that the strains were placed in the same cluster with the reference strain of *D. zeae*. Host range assessment showed positive soft rot symptoms in *Aloe vera*, chinese cabbage, dragon-fruit, eggplant, lettuce, and welsh onion that have never been reported before.

Keywords: Characterization, *Dickeya zeae*, host range, pineapple, soft rot symptom

INTRODUCTION

The genus *Dickeya*, previously known as *Erwinia chrysanthemi*, is a facultative aerobic or anaerobic phytopathogenic bacterium that causes soft rot diseases on many crops. The genus *Dickeya* comprises of six species, namely *D. dianthicola, D. dadantii, D. zeae, D. chrysanthemi, D. dieffenbachia* and *D. paradisiaca* (Samson et al. 2005), and nine biovars which largely correspond to biovar classification of *E. chrysanthemi* (Ngwira and Samson, 1990, Samson et al. 2005). The genus *Dickeya* has undergone several taxonomic revisions and recently the species of *D. dieffenbachiae* has been revised as *D. dadantii* subsp. *dieffenbachiae* (Brady et al. 2012). Some isolates of *Dickeya* from European potatoes have been determined as a new species, such as *D. solani* (van der Wolf et al. 2014). Another new species of *Dickeya* isolated from waterways in the UK and Finland was identified as *D. aquatica* (Parkinson et al. 2014). In addition, three strains of *Dickeya* isolated from pear bleeding cancer in China were proposed as a novel species as *D. fangzhongdai* (Tian et al. 2016). Therefore, at present, there are eight species in the genus *Dickeya* including *D. dianthicola, D. dadantii, D. zeae, D. chrysanthemi, D. paradisiaca, D. solani, D. aquatica* and *D. fangzhongdai*. Among the 11 species, two of them (*D. zeae* and *D dadantii*) were identified as the causative agents of pineapple soft rot (Samson et al. 2005) that better known as bacterial heart rot disease of pineapple.

Bacterial heart rot and fruit collapse disease on pineapple was first reported in Malaysia (Johnston, 1957). The disease is economically very important to pineapple producers and now has been spread to Costa Rica, Brazil, Philippines (Rohrbach & Johnson, 2003), and Hawaii (Kaneshiro et al. 2008). The bacterial pathogen, however, has not been clearly identified. Bacterial pathogen firstly found in Malaysia was reported as *E. carotovora* (Johnston, 1957), but later was identified as *E. chrysanthemi* (Lim, 1974). The bacterial pathogen found in Philippines and Hawaii was also identified as *E. chrysanthemi* (Kaneshiro et al. 2008). Since *E. chrysanthemi* has been reclassified into the genus *Dickeya* (Samson et al. 2005), the pathogen of bacterial heart rot and fruit collapse of pineapple found in Malaysia was then reported as *Dickeya zeae* or *Dickeya* sp. (Ramachandran et al. 2015). The symptoms observed in infected pineapples consisted of initial necrosis followed by a collapse of the tissue. Detailed studies of the morphology and physiology, as well as pathogenicity of *Dickeya* (*E. chrysanthemi*) isolated from pineapple and

several other crops, have been reported from several pineapple producing countries (Avrova et al. 2002; Lim 1974; Kaneshiro et al. 2008; Ramachandran et al. 2015) but not from Indonesia. In this paper, the bacterial heart rot and fruit collapse disease will be referred to bacterial soft rot.

In 2013, bacterial soft rot symptoms were observed in the harvested pineapple in East Lampung, Indonesia. The disease symptoms were very similar to the bacterial heart rot or soft rot and fruit collapse reported by Kaneshiro et al. (2008). This finding should not be ignored since the disease might have been introduced or even spread in Indonesia in several ways. The pathogen may have infected the previously imported crowns and was cultivated in Lampung then developed and spread in the pineapple field. Since there is no publication of this bacterial disease from any pineapple producing areas in Indonesia, so it is difficult to collect information on the disease intensity and yield loss caused by the disease.

Field observation showed that the disease symptoms on pineapple appeared as water-soaked lesions on the upper surface of leaves arising from the base. In general, the symptoms were similar to the bacterial heart rot of pineapple caused by *Erwinia chrysanthemi* found in Hawaii (Kaneshiro et al. 2008) or caused by *Dickeya zeae* or *Dickeya* sp. found in Malaysia (Ramachandran et al. 2015). The previous study by Prasetyo and Aeny (2014) showed that the symptoms, pathogenicity, and morphological characteristics suggested that the pineapple soft rot and fruit collapse in Lampung was caused by *E. chrysanthemi*, that later on known as *D. zeae* (Aeny et al. 2018). Due to the serious threat of the bacterial soft rot disease to pineapple production, the suspected *Dickeya* sp. isolated from diseased pineapples in East Lampung, Indonesia must be accurately characterized and identified. The fact that its taxonomy remains unstable, it might cause difficulties in understanding disease management. This study aims to reveal the identity of the bacteria causing soft disease using a biochemical and molecular technique based on sequence analysis of 16S rDNA, *recA*, and *dnaX*, and to assess the host range. The result of this study will provide precious information on the species identity of the causative agent of pineapple soft rot in Lampung, Indonesia as well as various crops that may act as alternative hosts.

MATERIALS AND METHODS

Isolation of the pathogen

The pineapple leaves showing blister-like lesion symptoms were collected from the pineapple field in 2015. Leaves were surface-disinfected using 70% ethanol. Small portions of leaf tissues were cut at the boundary of healthy and infected leaf tissue and placed in a microtube (1.5 mL) containing 500 µL of sterile distilled water. The tissues were then submerged and macerated using tweezers and left for 10 minutes. Subsequently, a loopful of the suspension was streaked onto yeast peptone agar (YPA) (Suharjo et al. 2014) and incubated for 48 hours at room temperature (28°C). The selected isolates were then sub-cultured to obtain pure culture before being stored in skim milk agar (Suharjo et al. 2014) at-40°C for further investigation.

Affirming that the obtained bacterium was the causative agent of soft rot disease, so the bacterial inoculation was carried out to the healthy pineapple plants. Three healthy pineapple plants were planted in polybags and placed in the glasshouse. The bacterial suspension (~10^8 CFU/mL) was prepared and injected into healthy 4-month-old plants using a sterilized syringe in the midsection of the leaf (Kaneshiro et al. 2008; Ramachandran et al. 2015). The inoculated plants were covered with transparent plastic bags to maintain relative humidity and were removed after three days. As for the negative control, the pineapple plants were injected with sterile distilled water. Symptoms of infection were observed and recorded every day for 3 weeks. Re-isolation and characterization of the bacterial strains were conducted to fulfill Koch's Postulates.

Phenotypic characterization

Gram reaction was investigated by the KOH staining method using 3% KOH (Suslow et al. 1982). Fluorescent pigment production was investigated on King's B medium (King et al. 1954). Oxidation and fermentation test was performed using the medium described by Hugh and Leifson's (1953). Potato soft rot test, lecithinase test, and hydrolysis of casein test were conducted based on Lelliot et al. (1966). Arginine Dehydrolase (ADH) Moeller (Himedia, India) (with the addition of a 1% L-Arginine hydrochloride) test was performed based on Dickey (1979). Growth capability at 5% NaCl was performed based on the methods described by Dye (1968). The utilization of 11 organic compounds as a sole source of carbon was tested on the modified Ayers Medium (Society of American Bacteriologist, 1957), with 0.1% (w/v) organic compounds incorporated. A positive reaction was assessed when bacterial growth was observed within 21 days at 27 °C. Growth capability at 36, 37, 39, 40 and 41°C was tested using the YP broth medium.

DNA extraction and PCR amplification

Bacteria were inoculated into 5 mL yeast peptone (YP) medium (Suharjo et al. 2014) and cultured in a shaking incubator (185 rpm) at 27°C overnight. The bacterial cells were harvested by centrifugation (14,000 rpm for 10 min). The DNA was extracted from the bacterial cells using the cetyltrimethylammonium bromide method (Ausubel et al. 2003). For molecular analysis, the DNA was used at the concentration of ~ 1 µg/µL.

PCR amplification was performed using 25 µL total volume of the mixture 16SrDNA, *recA*, and *dnaX* using MyTaq™ Red Mix (Bioline, USA) according to manufacturer's instruction. The PCRs were conducted using SensoQuest (Germany) thermocycler machine. The PCR was carried as follows: 1 cycle of an initial denaturation at 94°C for 5 min, 30 cycles consisted of denaturation at 94°C for 1 min, annealing at 58°C (16S rDNA), 59°C (*dnaX*) or 56°C (*recA*) for 1 min, primer extension at 72°C for 1 min, and final extension at 72°C for 5 min. The primers used in this study are listed in Table 1.

Table 1. Polymerase chain reaction (PCR) primer sequences used in this study

Locus	Primer	Sequence (5'-3')	Reference
16S rDNA	fD1	CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG	Weisburg et al. 1991
	rP2	CCCGGGATCCAAGCTTACGGCTACCTTGTTACGACTT	
dnaX	dnaXf	TATCAGGTYCTTGCCCGTAAGTGG	Sławiak et al. 2009
	dnaXr	TCGACATCCARCGCYTTGAGATG	
recA	RS1	GGTAAAGGGTCTATCATGCG	
	RS2	CCTTCACCATACATAATTTGGA	Suharjo et al. (2014)

DNA sequencing

The PCR products of 16S rDNA, *dnaX* and *recA* were electrophoresed in 0.5% agarose gels containing ethidium bromide (10 mg ml^{-1}) with Tris-Boric Acid-EDTA (TBE) buffer (pH 8.0) at 50 Volt for 70 min. The result was visualized under DigiDoc UV transilluminator (UVP, USA). The PCR products of 16S rDNA, *recA*, and *dnaX* were sent to 1st Base, Malaysia for sequencing.

Phylogenetic analysis

The sequencing results were then analyzed using BioEdit for Windows program ver. 7.2.6 (Hall, 1999). The phylogenetic tree was constructed based on sequences of 16S rDNA, *recA* and *dnaX* using the neighbor-joining method (Jukes and Cantor model) with MEGA7 for Windows (Kumar et al. 2016). Sequence data of *Dickeya* species reference strains were obtained from NCBI GenBank (https: //www.ncbi.nlm.nih.gov/).

Host range test

Seedlings of corn, rice aloe vera, celery, chrysanthemum, spring onion, dendrobium were inoculated by stabbing the bacterial suspensions (~10^8 CFU/mL) into their stem. Slices of bulb onion; detached leaves of bok choi, cabbage, Chinese cabbage, and curly lettuce; fruits of avocado, bean, coyote, cucumber, dragon fruit, guava, long bean, tomato, and watermelon; and tuber of garlic and carrot were also inoculated using the stabbing method. The inoculated plant parts were placed in plastic boxes (40 x 40 x 60 cm) covered by transparent plastic and kept for 48 hours to maintain moisture. Observations were conducted every day for a week to record the symptoms indicated by color-changing (necrotic) and soft rot symptoms on the inoculated plant tissues.

RESULTS AND DISCUSSION

Pathogen characteristics

Bacterial isolation from pineapple leaves with blister-like lesion symptoms resulted in ten bacterial isolates with the code of N-Unila 1 to N-Unila 10 that the colony characteristics as follows: round, white, convex, the opaque colony on YPA medium at 24 hours after inoculation. At 48 to 72 hours after inoculation, the colony shape turned to nearly round with irregular edge. Microscopic observations showed that the cells were straight rods with rounded ends, occurred singly or in pairs, and non-spore-forming (Figure 1). Two representative strains (N-Unila 5 and N-Unila 10) were selected for further investigation.

The representative strains were capable of infecting plant tissue and caused symptoms on the inoculated pineapple plants within 7 days after inoculation. The disease symptoms were initiated as a small water-soaking lesion around the stabbed point, blotted and light brown discoloration. The water-soaking lesions enlarge and spread further from the inoculated area and formed dark infection border. Further symptoms exhibited color changes from light brown to dark brown with a blisters-like appearance on the inoculated leaf (Figure 2). Plant leaves inoculated with sterile distilled water as control, did not show disease symptoms. Reisolated bacteria from the infected leaves also showed disease symptoms on the new inoculated plants and confirmed that the Koch's postulate was fulfilled.

Based on the biochemical tests, the representative strains (N-Unila 5 and N-Unila 10) were placed in phenon 1 (biovar 3 and 8) which corresponds to *D. zeae* and *D. dadantii* (Table 2). These isolates could not grow in the presence of 5% NaCl but survived at 41°C. The isolates were Gram-negative, ADH Moeller negative, fermentative, soft rot positive on sliced potato tuber and lecithinase positive. They utilized several different sugars such as Myo-inositol, M-tartrate, mannitol, L-tartrate, lactose, glycerol, D-melibiose, D-arabinose, citrate, and cis-aconitic acid but did not utilize starch, 5-ketoglucanate, L-ascorbic acid, inulin, folic acid, D-raffinose and D-tartrate as a sole carbon source.

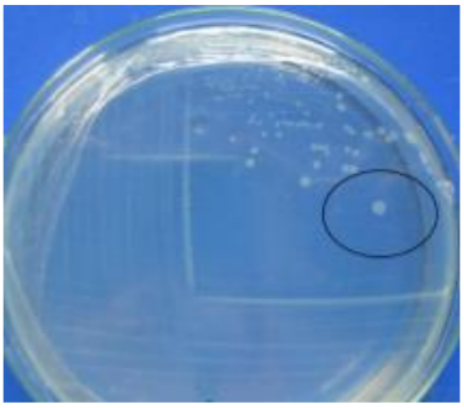

Figure 1. Colonies of *Dickeya* isolated from the pineapple plant

Figure 2. Symptoms of disease at seven days after artificial inoculation (*left*) and blister-like symptom in the pineapple field (*right*)

Table 2. The phenon characteristics of tested strains

Test	Tested strains (n=2)	Phenon 1	Phenon 2	Phenon 3	Phenon 4	Phenon 5	Phenon 6
Lechitine	+	+	+	+	+	+	-
ADH Moeller	-	d (15)	-	-	+	d (69)	-
Casein	nt	+	d (75)	d (80)	+	d (75)	-
Utilization of :							
D-arabinose	+	+	-	+	-	-	+
D-tartrate	-	-	d (75)	-	-	+	+
Inulin	-	-	-	-	+	d (88)	-
Lactose	+	+	d (75)	-	d (20)	-	d (17)
Cis-aconitic acid	+	+	-	d (80)	d (20)	-	-
D-melibiose	+	+	+	-	+	d (44)	d (83)
D-raffinose	+	+	+	-	+	d (44)	d (83)
5-ketogluconate	-	-	-	d (20)	-	-	+
Mannitol	+	+	+	+	+	+	-
M-tartrate	+	+	d(25)	-	-	+	+
Myo-innositol	+	+	+	d (80)	+	+	-
Growth at 39 °C	+	+	+	+	+	-	d (83)
Species name	*D. dadantii* + *D. zeae*	*D. dadantii* + *D. zeae*	*D. chrysanthemi* bv. *parthemi*	*D. dieffenbachiae*	*D. chrysanthemi* bv.*chrysanthemi*	*D. dianthicola*	*D. paradisiaca*

Note: nt: not tested; + : 90-100% positive;-: 90-100% negative; d(n) : percentage of positive strains. Phenon characteristics were described based on Samson et al. (2005)

Phylogenetic analysis based on the sequence of 16S rDNA, *recA*, and *dnaX*

BLAST analysis revealed that the DNA sequences of the three genes of the strains (N-Unila 5 and N-Unila 10) were 99% identical to those of *D. zeae* sequences available in the GenBank databases. Seventy-eight sequences of reference strains (including the type strain) of the known *Dickeya* species (16S rDNA; 20 sequences, *recA*; 29 sequences and *dnaX*; 29 sequences) for phylogenetic analysis were retrieved from NCBI GenBank. *E. coli* were used as outgroup control. Based on 16S rDNA sequences analysis (Weisburg et al. 1991), it revealed that the tested strains (N-Unila 5 and N-Unila 10) were placed in the same group with the reference strains of *D. zeae* (MAFF311098 (corn), acc. no. AB713546; MAFF106502 (rice), acc. no. AB713560; and SUPP410 (Setaria), acc.no. AB713536) (Figure 3). The result of *recA* corresponded to those results of 16S rDNA sequence analysis. The strains were in the same group with the type strain of *D. zeae* (NCPPB2538, acc. no. FJ216967) as well as other reference strains of *D. zeae* (MAFF311098 (corn), acc. no. AB713664; MAFF106502 (rice), acc. no. AB713671; SUPP410 (Setaria) acc. no. AB713693) (Figure 4). The same result was obtained based on the sequence analysis of *dnaX*. The strains were in the same group with the type strains of *D. zeae* (IPO2131 acc. no. GQ904764) as well as other reference strains of *D. zeae* (MAFF311098 (corn) acc. no. AB713581; MAFF106502 (rice) acc. no. AB713593, SUPP410 acc. No. AB713596) (Figure 5). These phylogenetic studies supported and confirmed the result of biochemical tests leading to the conclusion that the bacterial pathogen of soft rot disease of pineapple in East Lampung Indonesia was *D. zeae*.

Host range of *D. zeae* isolated from pineapple

Isolated bacteria from pineapple that inoculated to several plant species showed the bacteria were capable of infecting most of the inoculated plant species. Out of 25 plant species within 16 families, 17 species positively infected at 24-72 hours after inoculation (11 species in 10 dicot plant families and 6 species in 5 monocot plant families) (Table 3). Among those tested plants, inoculated *Aloe vera* showed the most severe symptoms at one week after inoculation (Figure 6). However, inoculated avocado fruit, banana, carrot, cabbage, coyote, cucumber, kalanchoe, and rice did not show any symptom.

Table 3. Inoculated plant species and their reactions as rot symptoms

Plant family	Name of species	Common name	Inoculated parts	Reaction*)
Apiaceae	*Apium graveolens*	Celery	Stem	+
	Daucus carota	Carrot	Tuber	-
Asteraceae	*Chrysanthemum indicum*	Chrysanthemum	Leaf	+
	Lactuca sativa	Curly lettuce	Leaf	+
Asphodelaceae	*Aloe vera*	Aloe vera	Leaf	+
Brassicaceae	*Brassica oleracea*	Cabbage	Leaf	-
(Cruciferae)	*Brassica chinensis*	Chinesse cabbage	Leaf	+
	Brassica rapa	Bok choi	Leaf	+
Bromeliaceae	*Ananas comosus*	Pineapple	Leaf	+
Cactaceae	*Opuntia littoralis*	Cactus	Leaf	+
	Hylocereus undatus	Dragon fruit	Fruit	+
Crassulaceae	*Kalanchoe pinnata*	Kalanchoe	Leaf	-
Cucurbitaceae	*Cucumis sativus*	Cucumber	Fruit	-
	Sechium edule	Coyote	Fruit	-
Fabaceae	*Phaseolus vulgaris*	Green bean	Pod	+
Lauraceae	*Persea gratissima*	Avocado	Fruit	-
Liliaceae	*Allium cepa*	Onion	Tuber	+
	Allium fistulosum	Welsh onion	Tuber	+
Myrtaceae	*Psidium guajava*	Guava	Fruit	+
Musaceae	*Musa paradisiaca*	Banana	Leaf, fruit	-
Orchidaceae	*Dendrobium* sp.	Orchid	Leaf	+
Poaceae	*Oryza sativa*	Rice	Stem	-
	Zea mays	Corn	Stem	+
Solanaceae	*Solanum lycopersicum*	Tomato	Fruit	+
	Solanum melongena	Eggplant	Fruit	+

Note: *) +: a symptom of necrotic, rot, or changing of tissue color; -: no symptom

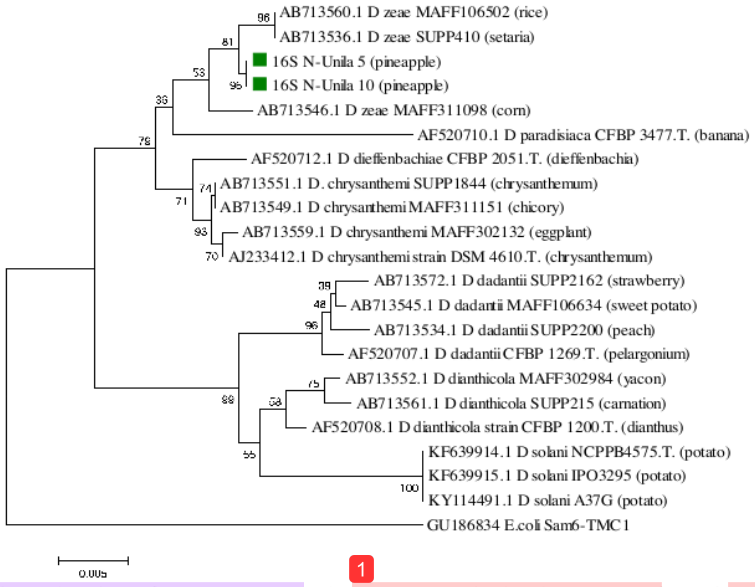

Figure 3. Phylogenetic tree based on 16S rDNA sequence. The tree was rooted using the sequence of *E. coli* strain Sam6-TMC1 (Acc no. GU1868340). The type strain of *Dickeya* species were also included. Reference strains used in this study were collected from the study of Suharjo et al. (2014) and van der Wolf et al (2014). ■: *Dickeya* sp. used in this study.

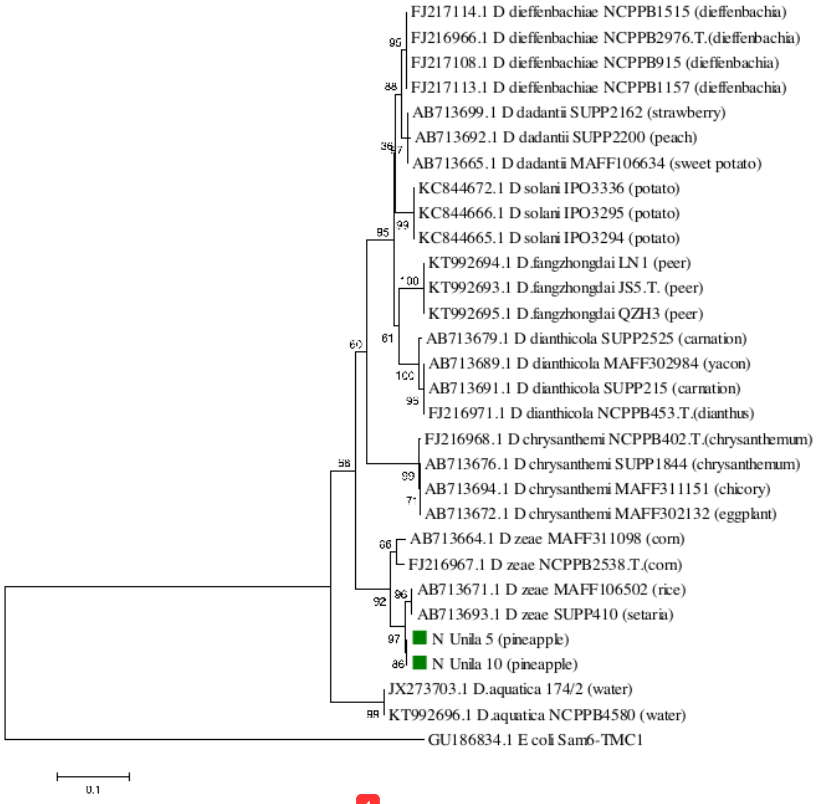

Figure 4. Phylogenetic tree based in the *recA* gene sequence. The tree was rooted using the sequence of *E. coli* strain Sam6-TMC1. The tree was rooted using the sequence of *E. coli* strain (GU186834). The type strain (T) of *Dickeya* species was also included. Reference strains used in this study were collected from the study of Suharjo et al. (2014), Parkinson et al. (2014), van der Wolf et al. (2014) and Tian et al. (2016). ■: *Dickeya* sp. used in this study.

Discussion

Based on the phenotypic characteristics, *Dickeya* spp. was divided into nine biovars (Ngwira and Samson, 1990) and six phena (phenotypic groups) (Samson et al. 2005). Phenon 1 (all the strains belong to biovar 3 and 8) resemble *D. zeae* or *D dadantii*, phenon 2 (all the strains belong to biovar 6) and phenon 4 (all the strains belong to biovar 5) resemble *D. chrysanthemi*, phenon 3 (all the strains belong to biovar 2) resemble *D. dieffenbachiae*, phenon 5 (all the strains belong to biovar 1, 7 and 9) resemble *D. dianthicola* and phenon 6 (all the strains belong to biovar 4) resemble *D. paradisiaca*.

Since the species of *Dickeya* have great variations and are closely related (Nassar et al. 1996), so identification using only phenotypic characteristics or single gene is not recommended (Kolbert et al. 1999; Marrero et al. 2013). Ngwira and Samson (1990) and Samson et al. (2005) reported that two species of *Dickeya*, namely *D. zeae* and *D. dadantii* could not be differentiated using phenotypic characterization. These two *Dickeya* species belonged to phenon 1 (biovar 3 and 8). It has also been reported that a group of putative new species of *Dickeya* was also placed within biovar 3 (Suharjo et al. 2014; Parkinson et al. 2009; Slawiak et al. 2009). The time consuming and the high subjectivity on the reading of phenotypic characteristics became another problem. The possibility of mislabeling or mishandling is one of the main reasons that the use of a single gene for sequence analysis is really not enough for accurate identification. Therefore in this study, three different gene sequences (16SrDNA, *recA*, and *dnaX*) were used for phylogenetic analysis.

In 2005, *Dickeya* spp. was differentiated into 5 species, namely *D. dianthicola, D. dadantii, D. zeae, D. chrysanthemi* and *D. paradisiaca* based on host range test, phenotypic characteristics, molecular analysis as well as serological assay (Samson et al. 2005). Afterward, another three *Dickeya* species were proposed *i.e. D. solani* (van der Wolf et al. 2004), *D. aquatica* (Parkinson et al. 2014) and *D. fangzhongdai* (Tian et al. 2016). In 2012, Brady et al. (2012) reclassified *D. dieffenbachiae* into subspecies of *D. dadantii* and identified as *D. dadantii* subsp. *diefenbachiae*. Meanwhile, *D. dadantii* was renamed as *D. dadantii* subsp *dadantii*.

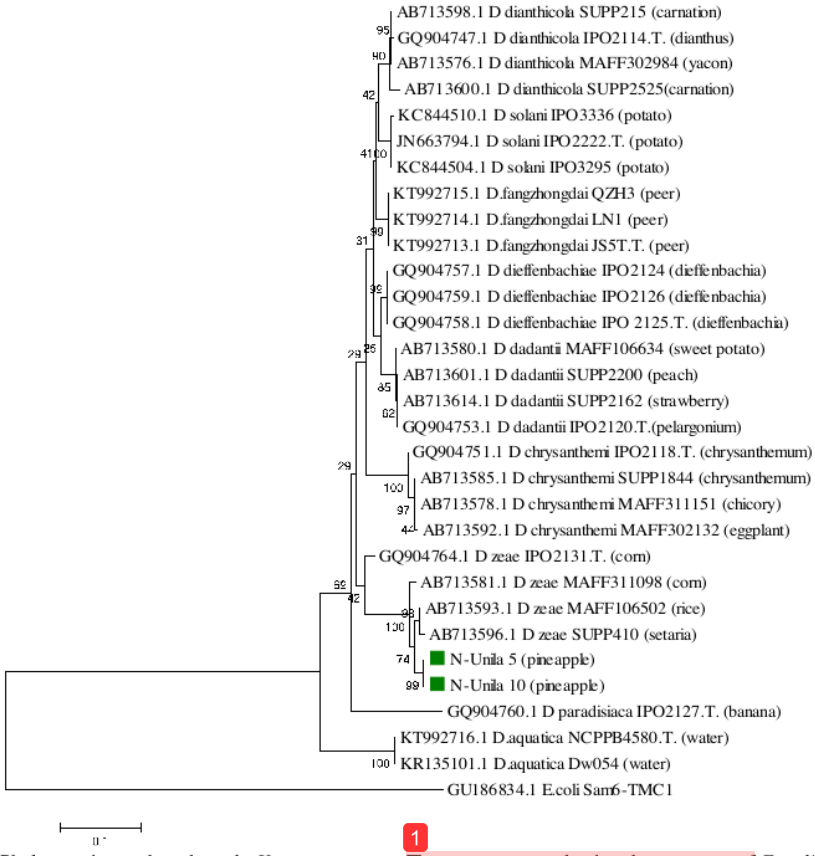

Figure 5. Phylogenetic tree based on *dnaX* gene sequence. The tree was rooted using the sequence of *E. coli* strain Sam6-TMC1. The tree was rooted using the sequence of *E. coli* strain (GU186834). The type strain (T) each of *Dickeya* species was also included. Reference strains used in this study were collected from the study Suharjo et al. (2014), Parkinson et al. (2014), van der Wolf et al. (2014) and Tian et al. (2016). ■: *Dickeya* sp. used in this study

Based on the biochemical tests, the two strains (biovar 3 and 8) were placed in the group of phenon 1 which belonged to *D. zeae* or *D. dadantii* (Samson et al. 2005). In order to reveal the species identity, another identification method using a multilocus sequence analysis was performed to draw an accurate conclusion. In this study, three different genes namely 16S rDNA, *recA*, and *dnaX* were used for bacteria identification. The 16S rDNA approach is considered as one of the most widely used standard techniques to infer phylogenetic relationships among bacteria but is sometimes insufficient to distinguish closely related species. The *recA* and *dnaX* genes have been proven as powerful markers for inferring bacterial phylogeny and have been used successfully to differentiate species of *Dickeya* (Samson et al. 2005; Slawiak et al. 2009; Parkinson et al. 2009; Suharjo et al. 2014; Zhang et al. 2014).

The result of BLAST analysis showed that the two bacterial strains contain 99% homologs to that of *D. zeae*. Based on the sequence analysis of 16S rDNA, *recA*, and *dnaX*, the two strains were placed in the group of *D. zeae*. Based on these findings, it is confirmed that the causative agent of pineapple soft rot in Lampung was the species of *D. zeae*. This report is considered as the first one on the pineapple soft rot disease caused by *D. zeae* in Indonesia.

Several fruit crops other than pineapple that cultivated in Lampung Province, such as guava and dragon fruit, were also considered as a potential host of the *Dickeya* soft rot pathogen. The tested fruits of guava and dragon fruit showed typical bacterial soft rot symptoms. As a bacterial soft rot pathogen, *Dickeya* sp. has been known as a pathogen of a wide range of tropical and subtropical crops, in the greenhouse and the field. Ma et al. (2007) reported that the genus *Dickeya* is composed of broad-host-range pathogens, including almost half of the orders of Angiosperm plants. The bacteria was also reported to cause soft rot disease in various plant species within 11 dicot families in 10 plant orders, and in 10 monocot families within 5 orders (Ma et al. 2007).

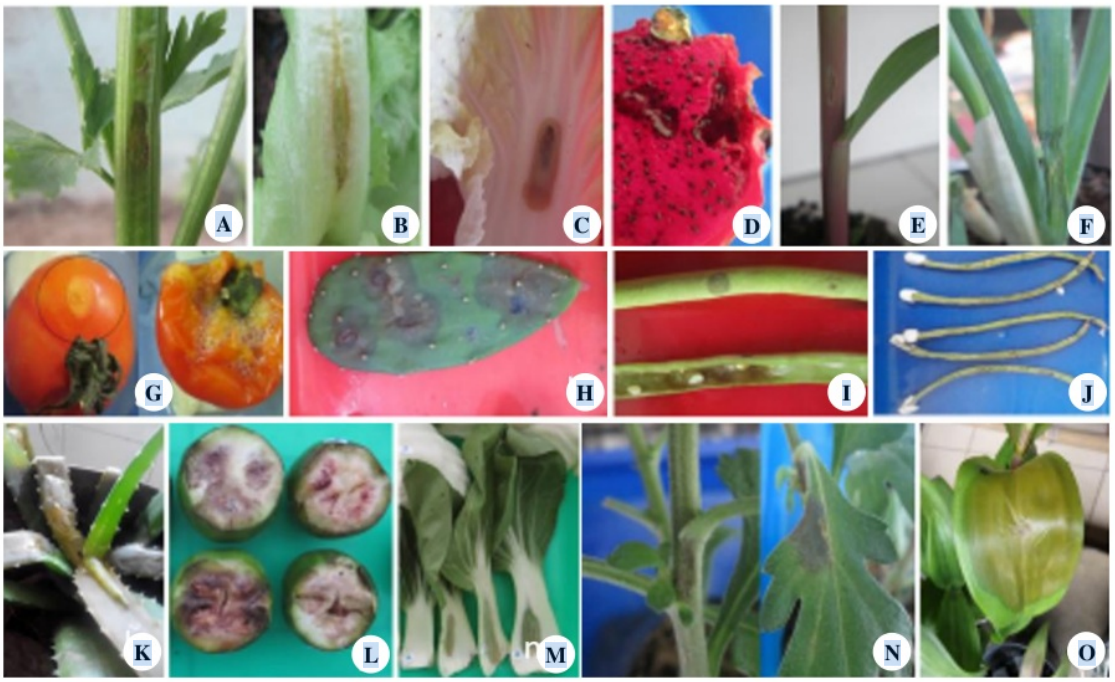

Figure 6. Positive symptoms produced by the strains on inoculated plants: A. Celery, B. Lettuce, C. Chinese cabbage, D. Dragon fruit, E. Maize, F. Welsh onion, G. Tomato, H. Cactus, I. Green bean, J. Long bean, K. Aloe vera, L. Eggplant, M. Bok choi, N. Chrysanthemum, and O. Dendrobium

The assessment of the host range in this study showed that *D. zeae* isolated from the pineapple field in Lampung, Indonesia was capable of infecting vegetables, ornamentals, and fruits but unable to infect banana. However, a study by Zhang et al. (2014) showed that *Dickeya zeae* was the causative agent of soft rot disease of banana in China. This difference was an interesting phenomenon showing variation in infection capability within *D. zeae* species that should be further investigated.

The information in the extent of the host ranges of *D. zeae* is very important to anticipate and prevent the spread of the disease, especially to other valuable crops. The occurrence of *D. zeae* in pineapple and other crops in Lampung should be considered as a warning for the production of pineapple as well as other crops. The pathogen might be a new latently infected strain or indigenous strain.

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