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CHARACTERIZATION OF BACTERIAL PATHOGEN CAUSING WILT AND LEAF BLIGHT ON CORN (*Zea mays*) BY PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR METHODS

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ABSTRACT

In 2011, we found a new bacterial disease characterized by wilt, dwarf and blight symptoms on sweet corn in Batu, East Java, Indonesia. The objective of this study is to characterize the causal agent of the disease. In this study, several assays were conducted, including hypersensitive response, pathogenicity, physiological and biochemical characteristics, PCR detection using two specific species primer pairs for *Pantoea stewartii* pv. *stewartii*, and homology analysis of 16S rRNA gene sequence. Four Gram-negative, non-motile, facultative anaerobic bacteria were isolated from the diseased tissue. Only two strains, BD1 and BB2, gave positive result in hypersensitive reaction, pathogenicity, and Koch's postulate assays. BB2 and BD1 strains also showed positive results in the PCR amplification using specific primers derived from the *P. stewartii* subsp. *stewartii* 16-23S gene region but showed negative result when using primers derived from *P. stewartii subsp. stewartii hrpS* gene region. Sequence of partial 16S rRNA gene of BD1 and BB2 showed highest homology at 96% to *P. stewartii* subsp. *stewartii* strain ATCC 8199 (NR. 044800.1). This results suggest that bacterial pathogens isolated from sweet corn in Batu were strains of *Pantoea* spp.

Keywords: *Zea mays*, *Pantoea* spp, wilt, leaf blight, PCR

INTRODUCTION

In Indonesia, corn has been an important crops for both the major feed for livestock and raw materials for food industry (Akil and Dahlan, 2010). More than 55% of domestic corn production is estimated to be used for livestock feed, 30% for food consumption, and the rest is for seed and other industrial needs (Kasryno *et al*., 2010). Production and harvest area of corn in Indonesia are arising during the last five years. In 2011, the corn production reached 17,629,033 tons yielded from 3.861.433 ha. East Java province is known to be the largest corn producer in Indonesia. In 2011, the corn production in East Java province was 5,443,705 tons yielded from 1,204,063 ha of production area (BPS, 2011).

Several plant diseases caused by fungal and bacterial pathogen have been reported causing losses of corn production in Indonesia. Recently, a new bacterial disease on corn found in several corn production area is wilt disease accompanied by leaf blight symptom. In 2009, this disease was reported in West Sumatra with the disease incidence ranging from 1%-15%. Based on the symptom and several physiological characteristic, the disease was predicted to be Stewart's wilt caused by *Pantoea stewartii* (Rahma and Armansyah, 2009). Recently, we found the similar bacterial wilt and leaf blight disease on sweet corn planted in Batu, East Java with the disease incidence ranging from 1% to 10%.

The most widely known of wilt and leaf blight disease of corn is Stewart's disease (Pataky, 2003). The causal agent of the disease is pathogenic bacteria known as *Bacillus stewartii*, *Bacterium stewartii*, *Erwinia stewartii*, *Pseudomonas stewartii*, *Pseudobacterium stewartii*, *Phytobacterium stewartii* or *Xanthomonas stewartii* (Brenner *et al*., 1984). In 1993, the bacteria were

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transfered to the genus of *Pantoea* to be *Pantoea stewartii* subsp. *stewartii* (Mergaert *et al*., 1993). The corn flea beetle *Chaetocnema pulicaria* Melsheimer (order: Coleoptera, family: Chrysomelidae) is known to be the primary vector for *P. stewartii* subsp. *stewartii* (Menelas *et al*., 2006). The potential risk of seed transmission is considered very important in international shipment of corn seeds hence more than 50 countries ban the importation of corn seeds unless it has been certified free of *P. stewartii* subsp. *stewartii* (Coplin *et al*., 2002; Michener *et al*., 2002).

Two phases of Stewart's wilt disease can occur on corn plants. The seedling wilt phase occurs when the young seedling plants are infected systemically, whereas the leaf blight phase occurs when the the infection occurs after seedling stage. The blight symptom appeared pale-green to yellow linear streaks with irregular or wavy margins develop parallel to leaf veins. These lessions become necrotic by age and on susceptible cultivars the lessions may extend to the entire length of the leaf. The leaves on the infected young plants usually show striped white or yellow (Lipps *et al*, 2003; Pataky, 2003). Systemic infection on corn seedling can cause high yield losses (Freeman and Pataky, 2001).

The study of the disease is still limited in Indonesia. Since its ability to infect corn plant in seedling stage, the disease is potential to be very destructive such as downy mildew caused by *Peronosclerospora maydis* which has been causing great loss of corn production in Indonesia. East Java province is the largest corn producer as well as the center of corn seeds production in Indonesia. Therefore, the presence of this disease can be a serious problem not only for corn production but also for healthy seed production.

This study aimed to characterize the bacterial pathogen causing wilt and leaf blight on corn found in Batu, East Java.

MATERIALS AND METHODS

The study was conducted at Plant Pathology laboratory of Plant Protection Department, Faculty of Agriculture, Brawijaya University, Malang, Indonesia and at laboratory of Mole-cular Biology of Biology Department, Faculty of Sience, Islamic State University, Malang, Indonesia, from September 2011 to March 2012.

Isolation and Identification of Bacterial Pathogen from Diseased Corn Plant

Bacterial pathogens were isolated from plant leaves and stalks showing wilt or blight symptoms collected on January 2011 from corn fields in Batu. Small sections of stalk or leaf tissues with indicated symptoms were cut aseptically from the margins of lesions and macerated in 1 ml of sterile distilled water for 30 minutes. Plant extracts were streaked onto Nutrient Agar (NA), incubated at 28ºC, and examined after 2 to 5 days for colonies appearance. Initial identification was performed i.e. the Gram staining, Hugh-Leifson assay, pigment production on YDC, and hypersensitive reaction (HR) on tobacco.

Pathogenicity and Koch's Postulate Assays

Pathogenicity assay on corn plants was performed by inoculation on sweet corn plants cultivar Jambore. Each bacterial strain was grown on NA plates for 48 h at 28ºC, and then each bacterial strain was suspended in sterile distilled water to obtain approximately 10^8 CFU/ml. Ten days old plants were inoculated using two methods: (i) suspensions of pathogenic bacteria were infiltrated into leaf tissues with a needle-less syringe and (ii) the suspensions were injected into plant stems. Inoculated plants were put in a chamber and maintained in room temperature with 90% relative humidity. After 2 days, the plants were transferred to greenhouse. Bacteria were re-isolated from symptomatic leaves or stems tissues and confirmed by observation of colony morphology on YDC, Gram staining, and Hugh-Leifson assay.

Physiological and Biochemical Assays

Isolates were assayed as described by Schaad *et al*. (2000) i.e. growth at 37°C; nitrate reduction; gelatin liquification; indole production; motility; utilization of citrate, malonate and tartrate; acid production from glycerol, lactose, maltose, mannitol, sorbitol, meso inositol, cellobiose and sucrose.

DNA Isolation

Genomic DNA was isolated from bacterial cells using alkali lysis method (Ausubel *et al*., 1996) with minor modification. The 24 hours cultured bacterial cells were harvested by centrifugation at 10.000 g for 5 minutes and re-

suspended in 525 µl TE buffer. Bacterial cells were then disrupted by adding 60 µl of 20% SDS and Proteinase K (200 µg/ml), and then incubated at 37°C for 60 minutes. The suspension was mixed with 100 µl 5N NaCl and 80 µl CTAB and incubated at 65°C for 30 minutes. 750 µl Chloroform Isoamyl Alcohol (CIAA) was added in the suspension, mixed thoroughly, and separated by centrifugation at 11.000 g for 10 minutes. Supernatant was transferred into new tubes and the equal volume of Phenol Chloroform Isoamyl-Alcohol (PCI) was added, mixed thoroughly and separated by centrifugation at 11.000 g for 10 minutes. The DNA from the supernatant was precipitated using equal volume of isopropanol and centrifugated at 12.000 g for 2 minutes. DNA precipitate was washed with 70% ethanol, re-centrifuged at 12.000 g for 2 minutes, dried on room temperature and re-suspended with 30µl TE buffer containing RNAase.

PCR Assay

For the determination of pathogenic *Pantoea,* two specific primer pairs designed for the detection of *P. stewartii* subsp. *stewartii* (Coplin *et al*., 2002) were used. Primers ES16 (5'-GCG AACTTGGCAGAG AT-3') and ESIG2c (5'-GCGCTTGCGTGTTATGAG-3') were derived from the sequence of 16S-23S rRNA/ITS region of *P. stewartii* subsp. *stewartii* whereas HRP1d (5'-GCACTCATTCCGACCAC-3') and HRP3r (5'-CGGCATACCTAACTCC-3') were derived from *hrpS* gene region of *P. stewartii* subsp. *stewartii*. The condition of PCR amplification were: 1 cycle at 95°C for 1 min, 55 cycles of: 30 s at 95°C, 30 s at 55°C, 60 s at 72°C, and 1 cycle of post extension at 72°C for 3 min. The PCR product was separated on 1.5% agarose gel at 1.5 V/cm, stained in ethidium bromide solution (0.5 µg/mL in TAE buffer) for 15 min, and subjected to Gel-Doc observation. The size of the expected amplicons were 0.92 kb (ES16/ESIG2c) and 0.9 kb (HRP1d/HRP3r) .

Analysis of Partial 16S rRNA Gene Sequence

The partial 16S rRNA gene fragment was amplified using the universal primers fD1(5′-AGAGTTGATCCTGGCTCAG-3′) and rD1(5′-AAGGAGGTGATCCAGCCGCA-3′) (Weisburg *et al*., 1991). DNA was amplified in 25 µl reaction volumes containing 4 µl template DNA, 12,5 µl Go Taq® Green Master Mix 2X from Promega, 2,5 µL of 100 pmol of each primer, and 3,5 µL DDW. The conditions of PCR amplification were 1 cycle at 95°C for 3 min; 40 cycles at 95°C for 1 min, at 55°C for 1 min, and at 72°C for 2 min; post extension at 72°C for 3 min. For the confirmation, the PCR product was separated on 1.5% agarose gel at 1.5 V/cm. The DNA fragment was stained in ethidium bromide solution (0.5 µg/mL in TAE buffer) for 15 min and subjected to Gel-Doc observation. The size of the expected amplicon was 1.5 kb.

The amplified PCR products were purified using ethanol/EDTA precipitation method. The sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequences were analyzed using Sequencing Analysis Software v5.31 (Applied Biosystems, USA). The GenBank/EMBL databases were used for homology search using the BLAST program that available in the website: http://blast.ncbi.nlm.nih.gov/Blast.cgi (National Center for Biotechnology Information, USA). Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 5 (Tamura *et al*., 2001).

RESULTS AND DISCUSSION

In this study we characterized the pathogenic bacterium causing wilt accompanied with blight symptoms on corn found in Batu area, East Java. The disease intensity varied from 1-10% of the plant population. The leaf blight symptom was typically light brown with irregular edges (Figure 1). Dwarf symptoms were found less than 5%, and the wilt symptoms were found on mature plants at approximately 50 days old. Blight symptoms were also found in younger plants. The wilted corn plants released bacterial mass from the cutting of rotted stalk. The characteristic of the symptoms mentioned above appeared to resemble the symptoms of Stewart's wilt disease of corn (Pataky, 2003).

Figure 1. Disease symptoms on corn observed in the field. A, leaf blight; B, stalk rot; C, dwarf

Bacterial Pathogen Isolation and Hypersensitive Assay

Four bacterial strains i.e. BB1, BD1, BB2, BB3 were isolated from leaves and stalks of corn showing disease symptoms. The bacteria were able to grow on NA medium in 24 hours at room temperatures. Colonies were circular, 1-2 mm, somewhat convex with flat edges. All isolates were Gram-negative rod, facultative an-aerobic and showed yellow colonies in YDC medium. All bacterial strains produced HR in tobacco leaves at 48 hours after infiltration.

Pathogenicity Test

When 10 days old seedlings were infiltrated with suspension of all bacterial strains, water soaked emerged in 2 days after inoculation. The symptoms changed into blight with irregular edges two days later. When the bacterial suspensions were injected into the stems of 10 days old corn plants, only BD1 and BB2 strains produced wilt symptoms within a week (Figure 2). These strains induced consistent symptom in corn plants similar to those observed in the field. Control plants inoculated with water did not develop any symptoms. Bacterial pathogens were successfully reisolated from the symptom of plants inoculated with strain BD1 and BB2. Colonies recovered diseased plants were also yellow, Gram negative, and facultative anaerobic.

Physiological and Biochemical Characteristics

The results of physiological and biochemical tests showed that strain BD1 and BB2 isolated from leaves and stem respectively had nearly similar characteristics, with the exception on the use of carbon from meso inositol assay. Characteristics of BD1 and BB2 strains resembled more to *P. ananatis*, but they had unique main characters similar to *P. stewartii subsp. stewartii* i.e. non-motile and were unable to produce indole. These results showed both BD1 and BB2 strains have physiological and biochemical characteristics quite differently from *P. stewartii* described by Schaad *et al.* (2000).

Both BD1 and BB2 strains showed non-motile suggesting that these strains are resembled to *P. stewartii* subsp. *stewartii*. However, other biochemical characteristics showed variable compared with those of *P. stewartii* subsp. *stewartii*. *P. stewartii* subsp. *stewartii* are historically described as aflagellated and non-motile bacteria (Pepper, 1967), the most distinguishing character of *P. stewartii* subsp. *stewartii* from other *Pantoea*. Transmission of *P. stewartii* subsp. *stewartii* in the field requires the feeding action of the pathogen bearing corn flea beetle, *Chaetocnema pulicaria,* which serves as overwintering host for *P. stewartii* subsp. *stewartii* (Pataky 2003). The existence of the corn flea beetle in Indonesia is still unknown.

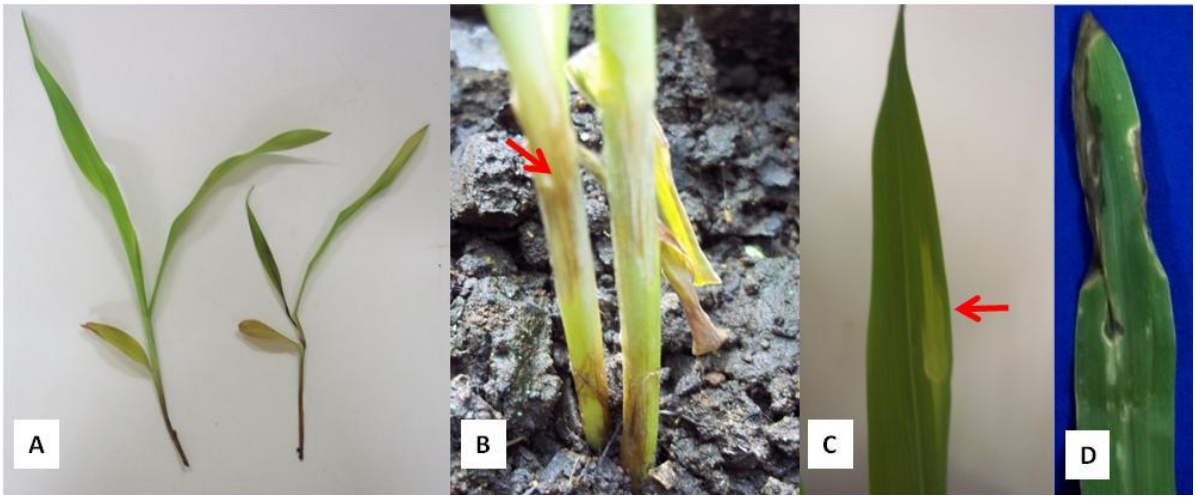


Figure 2. Symptoms on the pathogenicity test, A. wilt symptom (right) compared to control (left), B. watersoak appeared on stalk, C. water soak appeared on leaf, D. leaf blight

Detection of Bacterial Strains using Two Species-Specific Primer Pairs For *P. stewartii* subsp. *stewartii*

Primers ES16/ESIG2c were designed for the detection of *P. stewartii* subsp. *stewartii* to amplify the unique sequence of 16S to 23S rRNA intergenic transcribed spacer (ITS) region (Coplin *et al*.,2002). It is known that DNA sequences in the 16S to 23S intergenic spacer region exhibit a great deal of sequence and length variation. The variations in this region have been shown to be useful for differentiating species of prokaryotes (Barry *et al*., 1991).

Electrophoresis product using primer ES16/ESIG2c (Figure 3) indicated that 0,92 kb amplicons were detected on the BD1 and BB2 strains, but no amplicons were detected on the BB1 and BB3 strains. Positive control of genomic DNA of *P. stewartii* subsp. *stewartii* DM5262 strain also showed the amplicon, suggesting the positive result of both BD1 and BB2 strains.

The PCR assay using HRP1d and HRP3r primers derived from *P. stewartii* subsp. *stewartii hrpS* gene region showed that no amplicon was shown in all of 4 bacterial strains. Positive control of genomic DNA of *P. stewartii* subsp. *stewartii* DM5262 strain showed the amplicon, suggesting the negative result of the existence of *P. stewartii* subsp. *stewartii hrpS* gene in all 4 bacterial strains.

The ability of plant-pathogenic bacteria to elicit the hypersensitive response (HR) in resistant or non-host plants is correlated with their ability to cause disease in susceptible hosts. These capabilities are determined by hypersensitive response and pathogenicity (*hrp*) genes, which encode components of a protein secretion pathway, regulatory factors, and a number of secreted effector proteins. *P. stewartii* subsp. *stewartii* posses *hrpS*, one of *hrp* genes which involved in the HR elicitation in non host plants as well as pathogenicity in host plants (Lindgren, 1997)

Partial 16S rRNA Gene Sequence Analysis

The 16S rRNA gene sequence analysis is a powerful and accurate method for determining phylogenetic relationships (Barry *et al*., 1991). BLAST search using partial 16S rRNA gene sequences showed that BD1 and BB2 strains had the highest homology at 96% to *P. stewartii* subsp. *stewartii* strain ATCC 8199 (accession number NR_044800.1). These results suggest that both BD1 and BB2 strains are closed to species of *P. stewartii* subsp. *stewartii*. However, there is a consensus that two bacteria can be determined as same species if they shared as high as or more than 97% homology of 16S rRNA gene sequence. Thus, the BD1 and BB2 strains could not be considered as *P. stewartii* subsp. *stewartii*

Table 1. Physiological and biochemical characteristics of the bacterial strains isolated from corn, and published strains of *Pantoea stewartii* subsp. *stewartii, Pantoea ananatis* and *Pantoea agglomerans* (Schaad *et al.*, 2000)

Characteristic	BD1	BB2	*P.stewartii*	*P.ananatis*	*P. agglomerans*
			subsp. *stewartii*		(herbicola strains)
Growth at 37°C	+	+	-	+	+
Yellow pigmen on YDC	+	+	+	+	+
Indole production	-	-	-	+	V
Nitrate reduction	+	+	-	V	+
Gelatin liquification	+	+	-	+	+
Motility	-	-	-	+	+
Utilization of:					
* Citrate	+	+	-	+	+
* Malonate	-	-	-	-	+
* Tartrate	+	+	-	+	-
Acid production from:					
* Gliserol	+	+	-	+	-
* Lactose	+	+	-	+	V
* Mannitol	+	+	+	+	+
* Sorbitol	-	-	V	+	-
* Cellobiose	+	+	-	+	V
* Maltose	+	+	-	+	+
* Meso inositol	+	-	-	+	-
* Sucrose	+	+	+	+	+
V, between 21-79% of strains positive					

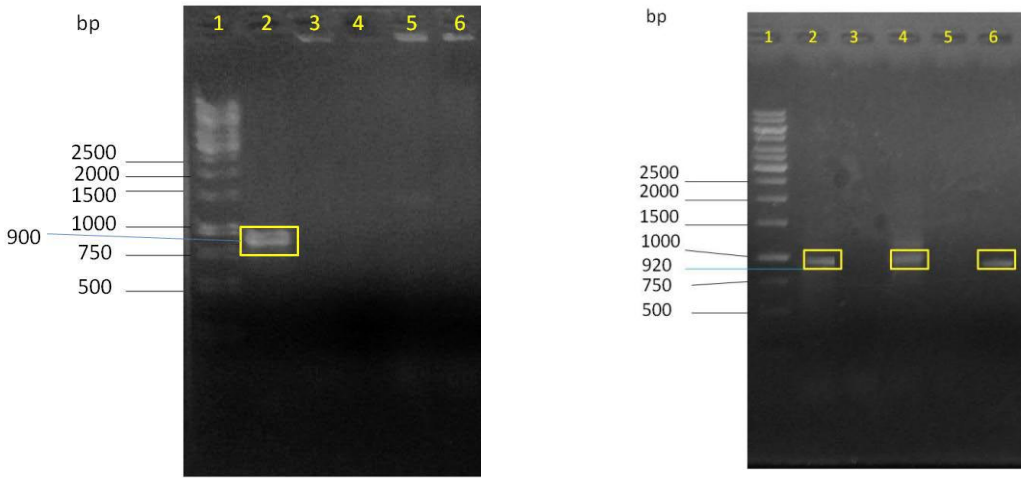
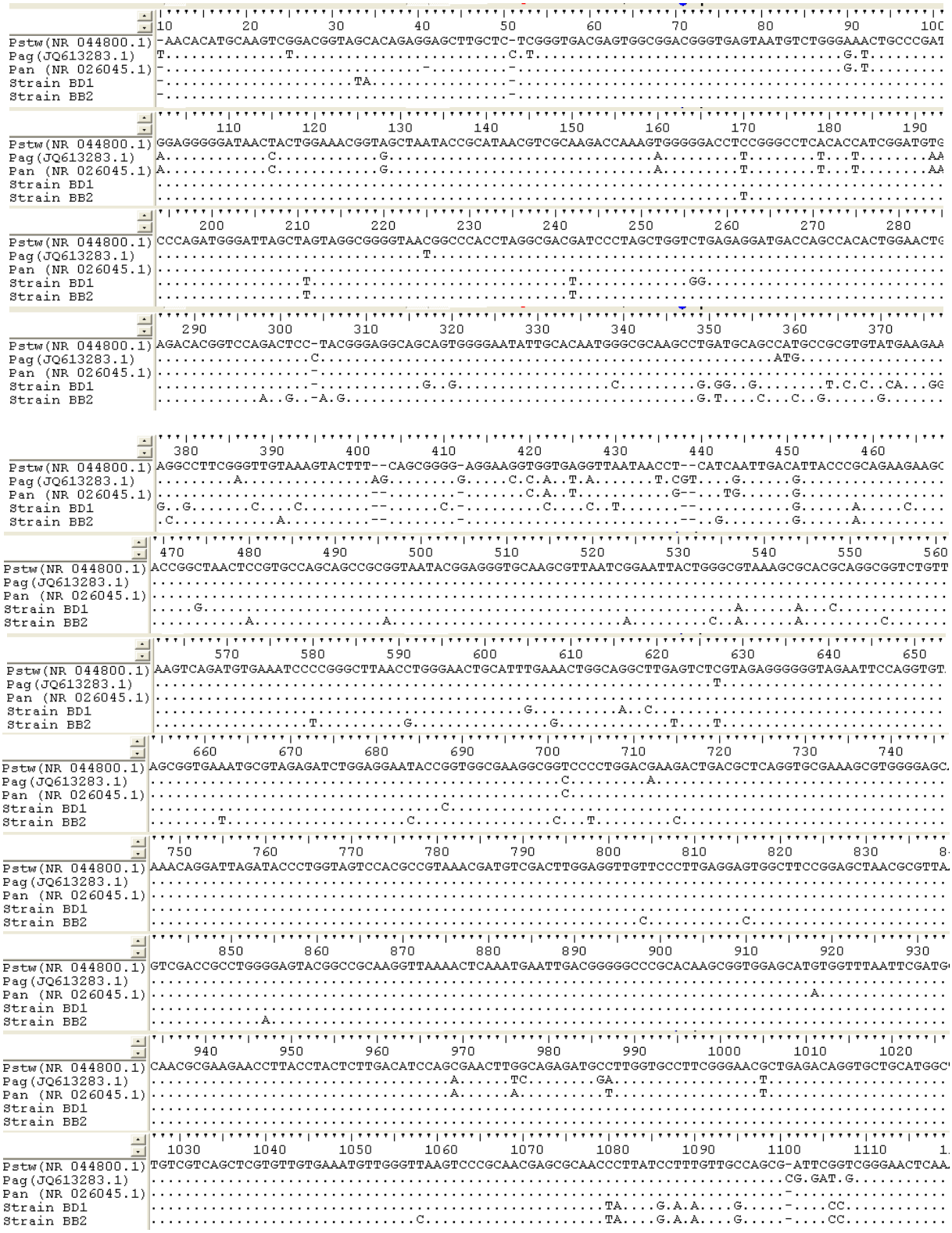


Figure 3. Gel electrophoresis visualization of PCR amplified bacterial DNA using primers ES16/ESIG2c (left) and *hrp*S (right); 1. 1kb DNA ladder; 2. DM5262; 3. BB1; 4. BD1; 5. BB3; 6. BB2


Pstw(NR 044800.1)
Pag(JQ613283.1)
Pan (NR 026045.1)
Strain BD1
Strain BB2

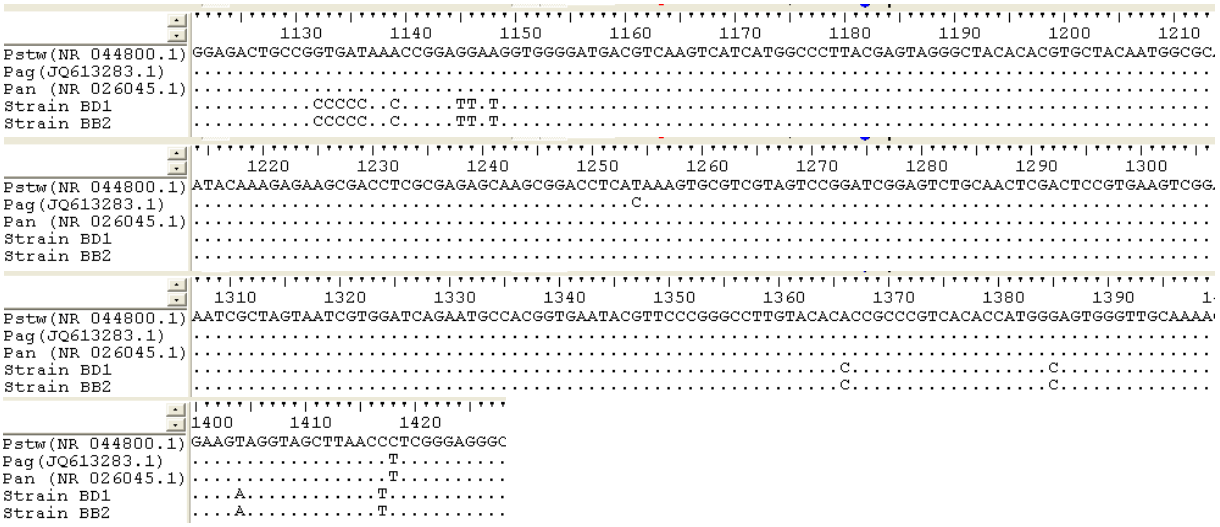


Figure 4. Sequence alignment of the 16S rRNA genes of *P. stewartii* subsp. *stewartii* (Pstw), *Pantoea ananatis* (Pan), *Pantoea agglomerans* (Pag), strain BD1, and strain BB2. Identical nucleotides are indicated by dotes, and deletions are indicated by dashes.

Multiple alignments of nucleotide sequences were performed using Clustal W program. The sequences were align with the nucleotide sequence of *P. stewartii* subsp. *stewartii* strain ATCC 8199 as well as those of *P. ananatis* and *P. agglomerans* which has close relationship to *P. stewartii* subsp. *stewartii*. The BD1 and BB2 showed high similarity to *P. stewartii* subsp. *stewartii*, although several bases have been changed (Figure 4).

The phylogenetic relationship derived from a neighbor-joining analysis of the pairwise comparison among the partial 16S rRNA gene sequences of both BD1 and BB2 strains with six sequences of well described species of the genus *Pantoea* is shown in Figure 5. *Escherichia coli*, *Erwinia chrysantemi*, and *Ralstonia solanacearum* were used as the out group taxons. The sequences of *P. ananatis, P. agglomerans, P. stewartii, E. coli*, *E. chrysantemi*, *R. solanacearum* were obtained from the GenBank/EMBL databases. The tree diagram demonstrated that the sequences of BD1 and BB2 are clustered in a group with *P. stewartii* subsp*. stewartii* supported by high bootstrap value of 100.

Overall, the results showed that BD1 and BB2 strain were non-motile similar to *P. stewartii* subsp. *stewartii*. However, physiological and biochemical traits of BD1 and BB2 strains showed variable to those of *P. stewartii* subsp. *stewartii*. In addition, PCR amplification assay with two types of specific species primer pairs for the detection of *P. stewartii* subsp. *stewartii* showed inconsistency. Homology of 16S rRNA gene sequences of BD1 and BB2 strains to *P. stewartii* subsp. *stewartii* were below 97%, a consensus value for the two bacteria considered same species. The ELISA test using AGDIA Kit for *P. stewartii* subsp. *stewartii* also showed negative result for both bacterial strains (data not shown). Taken together the results were not consistent to verify that BD1 and BB2 bacterial strains found in Batu is *P. stewartii* subsp. *stewartii*. Thus, we conclude that the bacterial strains are the unique strain of *Pantoea* spp. The surveillance and further researches on the disease are necessary to develop strategies to manage the disease.

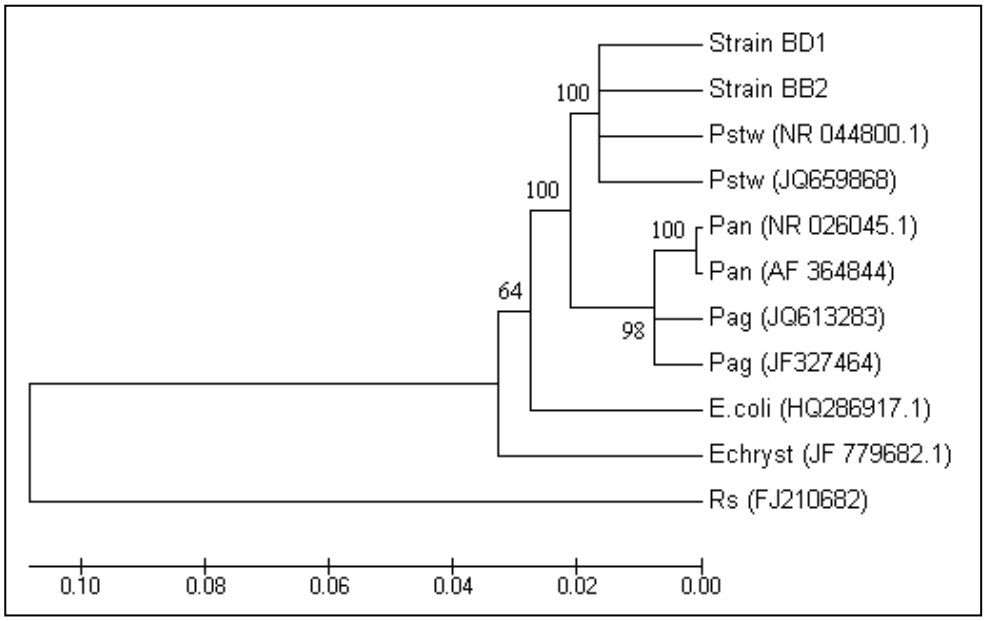


Figure 5. Phylogenetic tree showing the relationship among selected partial 16S rRNA gene sequences from *Pantoea* spp. and strains isolated from corn. The numbers at the nodes indicate the levels of bootstrap support based on data for 1,000 replicates. Accession numbers were showed in brackets. Bar represents genetic distance. *Pan = P. ananatis, Pag = P. agglomerans, Pstw = P. stewartii, E. coli = Escherichia coli*, *Echryst = E. chrysantemi*, *Rs = R. solanacearum*

CONCLUSION

Based on all tests performed, bacterial pathogen causing wilt and leaf blight on corn found in Batu was *Pantoea* spp. The bacteria had physiological and biochemical characteristics quite different from other published strains of *P. stewartii* subsp. *stewartii*, indicated that BD1 and BB2 are possibly unique strains of *Pantoea* spp. Further research on the disease is necessary to develop strategies to manage the disease as well as to prevent greater losses.

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REFERENCES

Akil, M. and H.A. Dahlan. 2010. Budidaya jagung dan diseminasi teknologi. *Dalam* Jagung: teknik produksi dan pengem-bangan. Litbang Deptan. Jakarta. p.192-204.

Ausubel F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl. 1996. Current protocols in molecular biology. John Wiley and Sons, New York.

Barry, T., G. Colleran, M. Glennon, L. Dunican and F. Gannon. 1991. The 16S/23S ribosomal spacer as a target for DNA probes to identify eubacteria. PCR Methods Appl. p.151-156.

BPS. 2011. Tanaman pangan. http://www.bps.go.id/tnmn_pgn.php

Brenner, D.J., G.R. Fanning, J.K. Leete Knutson, A. G. Steigerwalt and M. I. Krichevsky. 1984. Attempts to classify herbicola group-Enterobacter agglomerans strains by deoxyribonucleic acid hybridization and phenotypic tests. *Int. J. Syst. Bacteriol.* 34: 45-55.

Coplin, D.L., D.R. Majerczak, Y. Zhang, W.S. Kim, S. Jock and K. Geider. 2002. Identification of *Pantoea stewartii* subsp. *stewartii* by PCR and strain differentiation by PFGE. *Plant Dis*. 86: 304-311.

Freeman, N.D. and J.K. Pataky, J.K. 2001. Levels of Stewart's wilt resistance necessary to prevent reductions in yield of sweet corn hybrids. *Plant Dis*. 85: 1278-1284.

Kasryno, F., E. Pasandaran, Suyamto, M.O. Adnyana. 2010. Gambaran umum ekonomi jagung Indonesia. *Dalam* Jagung: teknik produksi dan pengembangan. Litbang Deptan. Jakarta. p. 474-497.

Lindgren P.B. 1997. The role of *hrp* genes during plant bacterial interactions. *Annual Review of Phytopath*. 35: 129-152.

Lipps, P.E., A.E. Dorrance and D.R. Mills. 2003. Stewart's bacterial wilt and leaf blight of corn. Ohio State University Fact Sheet. http://ohioline.osu.edu.

Menelas, B., C.C. Block, P.D. Esker and F.W. Jr. Nutter. 2006. Quantifying the feeding periods required by corn flea beetles to acquire and transmit *Pantoea stewartii*. *Plant Dis*. 90: 319-324.

Mergaert, J., L. Verdonck and K. Kersters. 1993. Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora)* and *Erwinia stewartii* to the Genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb, nov., Respectively, and description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Int. J. Sistematic Bacteriol.* 43(1): 162-172.

Michener, P. M., J. K. Pataky and D.G. White. 2002. Rates of transmitting *Erwinia stewartii* from seed to seedlings of a sweet corn hybrid susceptible to Stewart's wilt. *Plant Dis*. 86: 1031-1035.

Pataky, J.K. 2003. Stewart's wilt of corn. APSnet, American Phytopathological Society, St. Paul. MN, U.S.A. Published online.

Pepper, E. H. 1967. Stewart's bacterial wilt of corn. Monograph 4. American Phytopathological Society, St. Paul, MN, U.S.A

Rahma, H. and Armansyah. 2009. Penyebaran penyakit Stewart oleh bakteri *Pantoea stewartii* sebagai penyakit baru pada tanaman jagung (*Zea mays*) studi kasus di Pasaman Barat. Working Paper. Fakultas Pertanian. Univ. Andalas. http://lp.unand.ac.id/?pModule=penelitian&pSub=penelitian&pAct=detail&id=9&bi=2

Schaad, N.W., J.B. Jones and W. Chun . 2000. Laboratory guide for identification of plant pathogenic bacteria. Third Edition. Gail Hoover. Maryland. pp.372.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. molecular biology and evolution (submitted).

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane. 1991. 16S Ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*. 173(2): 697-703.