INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 2, p. 104-115, 2022

RESEARCH PAPER

OPEN ACCESS

Identification of Bacteria Causing Maize Wilt Disease in Tanah Laut Regency, South Kalimantan

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Key words: Biochemistry, Biomolecular, Pathogenic bacteria, Maize wilt.

http://dx.doi.org/10.12692/ijb/20.2.104-115

Abstract

Wilt disease is one of the problems in maize production caused by fungi and bacteria. Tanah Laut Regency, the center of maize production in South Kalimantan, also experienced this disease. Accurate identification of pathogens that cause wilt disease in maize in South Kalimantan, including in Tanah Laut District, has not been reported. Therefore, this study was conducted to identify the cause of wilt disease in maize. This research was carried out at the maize center in Tanah Laut Regency, South Kalimantan; the Phytopathology Laboratory, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru; and the Class I Agricultural Quarantine Laboratory, Banjarmasin. Collection and selection of bacteria isolated from sample plants were carried out to obtain pathogenic bacteria that cause wilt disease in maize and then tested biochemically and biomolecular. This study found 10 isolates of pathogenic bacteria that attack maize crops in Tanah Laut Regency. The ten bacteria belong to 4 genera, namely *Pantoea, Erwinia, Dickeya*, and *Pectobacteria*. Biomolecular tests found one isolate suspected to be *Pantoea stewartii*.

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Introduction

Maize has a vital role in Indonesia because of its multipurpose uses. Apart from being a raw material for food processing, maize is also beneficial as a feed ingredient and a source of export income. In the national economy, maize is the second largest contributor after rice. Indonesia's fresh maize export products are in fifth place, while processed maize exports are sixth (Purnawati, 2019).

Problems in maize production should be reduced because the maize industry plays a vital role in the Indonesian economy. One of the obstacles in maize production is the wilt disease caused by pathogens. This disease is caused by various types of fungi and bacteria with different symptoms and characteristics. For example, the fungus *Diplodia maydis* causes stalk rot called diplodia disease, and Gibberella stalk rot disease is caused by the fungus *Gibberella roseum* f.sp. *cerealis*. The most widely known fungus for causing wilting in maize is *Fusarium* sp. It causes fusarium stalk rot. The types of bacteria that cause this disease are *Pantoea stewartii* which causes Stewart wilt in maize, *Erwinia carotovora*, *E. chrysanthemi*, and *Pseudomonas syringae*. Losses caused by this disease vary widely from 5% to more than 50% (Sudjono, 2018).

In this study, we will focus on corn stem wilt caused by bacteria because one of the diseases that are quite deadly and causes damage up to 95% is caused by bacteria is Stewart wilt disease (Coplin *et al.*, 2002; EPPO, 2006; Goszczynska *et al.*, 2007; Roper, 2011; BBOPT, 2015). Stewart wilt is an important seed-borne disease of maize, as seeds are the most suitable means of transport for dispersal across natural boundaries (Neergaard, 1977). Currently, Stewart wilt is spread in many countries such as Europe (Austria), America (Bolivia, Brazil, Canada, Costa Rica, Guyana, Mexico, Peru, Puerto Rica, and the USA), Asia (China, India, Malaysia, Thailand, Vietnam) (Shurtleff, 1980).

In Tanah Laut Regency, South Kalimantan, one of the maize production centers, the pathogens attack that cause wilt disease can be found in almost every maizefield. The disease distribution, attack intensity, the extent of the attack, and the cause of the disease have not been reported. In-depth and accurate study of the causes of disease is the basis for accurate control. Therefore, this study aimed to identify the pathogens that cause wilt disease, especially those caused by bacteria on maize crops in Tanah Laut, South Kalimantan.

Material and methods

This study was conducted from July 2020 to March 2021. Samples of diseased plants were collected from all maize plantations in Tanah Laut District, South Kalimantan. Preparation of isolation, identification and several tests were carried out at the Phytopathology Laboratory, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru. PCR analysis was conducted at the Laboratory of the Class I Agricultural Quarantine Center Banjarmasin, South Kalimantan.

Sampling

Samples of diseased maize crops were taken from maize plantation centers in Tanah Laut Regency by observing the symptoms that appeared in the field. The target symptoms were soft rot in the stalk area and blight symptoms on the leaves.

Bacteria isolation

The isolates found consisted of isolates of fungi and bacteria. The fungal isolates were then eliminated. The bacterial isolates obtained were purified and identified. Isolation was performed by soaking the symptomatic plant parts sterilized with 70% alcohol and rinsed with sterile distilled water three times. The technique used was the streaking method by dipping the inoculation loop into the soaking water and then spreading it on Nutrient Agar (NA) media and incubating it for 24 hours. Each growing colony was purified.

Hypersensitivity test

The bacterial isolates aged 24 hours were harvested and then homogenized with a vortex mixer. The suspension was infiltrated on the underside of the tobacco leaf by injection without a needle. Incubation with cover was carried out for one week.

Pathogenicity test and koch postulates

The isolates aged 24 hours were harvested, homogenized, and then injected into the lower stalk of the 10 days-age maize crops. Maize crops were stored in a covered room to which newspapers and wet cloths were added to create a moist atmosphere and a suitable environment for the growth of pathogenic bacteria. Incubation was carried out for five days. Plants showing symptoms were then isolated again to prove that the causative pathogen was the cause of bacterial wilt disease in maize.

Bacteria identification

Gram Test, Anaerobic Growth Test, Test on YDC Media, Soft Rot Test, Lecithinase Test have been carried out based on the method Schaad *et al.* (2000).

Biochemical testing

Gelatin Liquefaction, Motility Test, Casein Hydrolysis, Acid Production and Utilization of Carbon Sources have been carried out based on the method of Schaad *et al.* (2000).

Biomolecular identification

DNA Extraction

DNA extraction was carried out using the method of the Manual book from *Quick*-DNA™ Miniprep Plus Kit Zymo Research, Instruction Manual Ver.1.3.2, revised on 4/16/2021. Genomic DNA was isolated from the bacterial cell using alkali lysis method of Ausubel *et al.*, 1996. PCR Amplification and Electrophoresis have been carried out based on the method of Suryani *et al.* 2012.

Observation parameter

Parameters observed were hypersensitivity in indicator plants, pathogenicity reactions in host plants, biochemical properties in bacteria, and bacterial genomes by PCR. All these series of tests were performed to identify bacteria to the genus level.

Data analysis

The research data was presented in the form of tabulated data and analyzed descriptively.

Results

Symptoms of disease in the field

Symptoms found in the field were a blight on leaves and soft rot on the stalk. Some plants experience rotting in the upper and lower stalk.

Another symptom was slimy rot on young plants. Symptoms of wilt accompanied by rot can be caused by bacteria *Pantoea* sp., *Dickeya* sp., and *Pectobacterium* sp. Observed symptoms in the field are presented in Table 1 and Fig. 1.

Table 1. The symptoms observed in the field.

No	Code	Sampling Location	Time	Symptoms
1	J1	Tajau Pecah Village, Batu Ampar District	July 21, 2020	Withered plants and soft rotten stalk
2	J2	Tajau Pecah Village, Batu Ampar District	July 21, 2020	Withered plants and soft rotten stalk
3	J5	Galam Sumsum Village, Bajuin District	July 21, 2020	Withered plants and soft rotten stalk
4	TL1	Ujung Baru Village, Bati Bati District	March 3, 2021	Dwarf plants, rotten and blackened lower stalk
5	TL2	Ujung Baru Village, Bati Bati District	March 3, 2021	Leaf blight and elongated yellowish spots
6	TL3	Plasma Pulau Sari, Tambang Ulang District	March 3rd, 2021	Slimy rot at the base of young leaves
7	TL4	Plasma Pulau Sari, Tambang Ulang District	March 3rd, 2021	Slimy rot at the base of young leaves
8	TL8	Bajuin Village, Bajuin District	March 3, 2021	Plants wither and lower stalk rot

Bacteria isolation

The previous bacterial isolates were obtained by removing the bacterial loops through immersion. Cloudy water that looks like smoke coming out of the stalk indicates a mass of bacteria in the symptomatic plant parts. This method is commonly used to identify a rot symptom in plants briefly and distinguish the disease's cause from bacteria and fungi. Bacteria that were successfully isolated and passed the selection had different colony morphological characteristics. The morphological data of isolated bacterial colonies are presented in Table 2 and Fig. 2.

Table 2. Colony morphology.

No	Isolate	Colony Form	Isolate Color	The margin of the Colony
1	TL12	Circular	Milky white	Entire
2	TL26	Circular	Yellow	Undulate
3	TL31	Circular	Yellowish white	Undulate
4	TL45	Circular	Yellow	Entire
5	TL410	Circular	Milky white	Entire
6	TL84	Circular	Milky white	Undulate
7	J15	Circular	Milky white	Entire
8	J29	Circular	Milky white	Entire
9	J218	Circular	Milky white	Entire
10	J518	Circular	Yellow	Entire

Hypersensitivity test

Medium-aged tobacco leaves were used to prolong the observation time. These leaves have the right size as a medium test. Of the 23 isolates tested, 17 bacterial isolates showed a positive hypersensitivity reaction. Chlorosis symptoms appeared on the 2nd-4th day and were followed by necrotic infiltration on the 5th-7th day (Fig. 3).

Pathogenicity test and koch postulates

The test plants showed wilting symptoms on the 3rd-5th day after injection. Rooting symptoms in the lower stalk around the injection site appeared in all samples. In addition, the leaves in symptomatic samples also showed a water soak. Following Koch's postulates, plants presenting symptoms were isolated again on NA media to prove that the symptoms were caused by the pathogen causing stalk rot disease in the maize crops (Table 3 and Fig. 4a and 4b). Koch's postulates are met if the bacteria growing around the plant cuttings have the same symptoms as the injected bacteria (Fig. 4c and 4d). It means that these bacteria are pathogenic bacteria that attack maize crops.

Table 3. The results of pathogenicity and Koch's postulates.

Isolate	Symptoms	The Koch's Postulates
TL12	Rotten stalk, water soak	Can be isolated
TL26	Water soak	Can be isolated
TL31	Rotten stalk	Can be isolated
TL45	Rotten stalk, water soak	Can be isolated
TL410	Rotten stalk, water soak	Can be isolated
TL84	Rotten stalk	Can be isolated
J15	Rotten stalk	Can be isolated
J29	Rotten stalk, water soak	Can be isolated
J218	Rotten stalk, water soak	Can be isolated
J518	Rotten stalk, water soak	Can be isolated

Bacteria Identification

Bacteria identification was carried out at the genus level. The test was carried out through 5 steps: the gram test, anaerobic growth test, yellow colony growth test on YDC, soft rot test, and lecithinase test. Identification of bacteria to the genus stage is presented in Table 4.

Table 4. Identification of bacterial genus.

Isolate	Test					Genus
	G	Anaerobic	YDC	SoftRot	Lesitinase	
TL12	-	+	-	+	-	*Pectobacterium*
TL26	-	+	-	-	NT	*Erwinia*
TL31	-	+	+	+	-	*Pectobacterium*
TL45	-	+	-	NT	NT	*Pantoea*
TL410	-	+	-	+	-	*Pectobacterium*
TL84	-	+	-	+	+	*Dickeya*
J15	-	+	-	+	+	*Dickeya*
J29	-	+	-	+	-	*Pectobacterium*
J218	-	+	-	+	-	*Pectebacterium*
J518	-	+	+	NT	NT	*Pantoea*

Note: NT, Not tested.

Biochemical testing

Based on the characteristic of the biochemical test of 10 isolates, two genera of Pantoea were found with different characteristics, namely isolates coded TL45 and J518. Based on the manual by Schaad (2000), when viewed from its characteristics, isolate TL45 has a tendency to species *Pantoea ananatis*, while isolate J518 is similar to *Pantoea stewartii*. It is estimated that these bacteria attack young maize plants and produce rotting symptoms on the stalk and bases of young leaves. Sample J5 showed indistinct symptoms, and there was only rot on the upper stalk of the maize. Observation of symptoms could not be performed because the sample was old and had passed the harvest period so that the plants were dry. Isolate J518 isolated from sample J5 is estimated to be *P. stewartii* bacteria. Based on the genus identification test, isolates TL12, TL31, TL410, TL29, and TL218 were categorized into the bacterial genus *Pectobacterium* sp. Based on several tests carried out on these five isolates, all of them had some similarities with *Pectobacterium brasiliense*. *Pectobacterium brasiliense* causes soft rot disease in various types of vegetables.

Table 5. Results of biochemical testing.

Testing	Isolate									
	TL12	TL26	TL31	TL45	TL410	TL84	J15	J29	J218	J518
Growth at 37°C	+	+	+	+	+	+	+	+	+	+
Liquification Gelatin	-	-	-	+	-	-	-	-	-	-
Motility Test	-	-	-	+	-	-	-	-	-	-
Casein Hydrolysis	-	NT	-	NT	-	-	-	-	-	NT
Carbon source test:										
Citrate	-	+	+	+	-	-	+	-	+	-
Tartrate	-	+	+	+	+	-	+	+	+	-
Meso Inositol	+	-	+	+	+	+	+	-	-	+
Lactose	+	-	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Maltose	+	-	+	-	+	+	+	+	+	+
Mannitol	+	+	+	-	+	+	+	+	+	+
Sorbitol	+	-	+	-	+	-	+	-	-	+

Note: NT, Not Tested.

Isolates TL84 and J15 had positive values in the lecithinase test and were included in the Dickeya category. These two isolates produced symptoms that matched the disease caused by bacteria of the genus *Dickeya* sp. The test using tartrate by Aeny *et al.* (2020) is divided into two types: D-tartrate and M-tartrate, where the results are only different on *D. Chrysanthemi* bv. *parthemi* which 75% of the strains were able to use D-tartrate, and 25% of the strains were able to use M-tartrate. The results of the biochemical tests on ten are presented in Table 5.

Biomolecular identification

Biomolecular identification begins with DNA extraction, which aims to separate DNA from other materials in cells, such as fats and proteins.

To ensure that pure DNA is obtained, electrophoresis is carried out. From the electrophoresis of 10 DNA extraction, DNA from 2 isolates was not obtained, namely TL84 and J29. This absence may be due to failure of DNA extraction or DNA damage prior to electrophoresis.

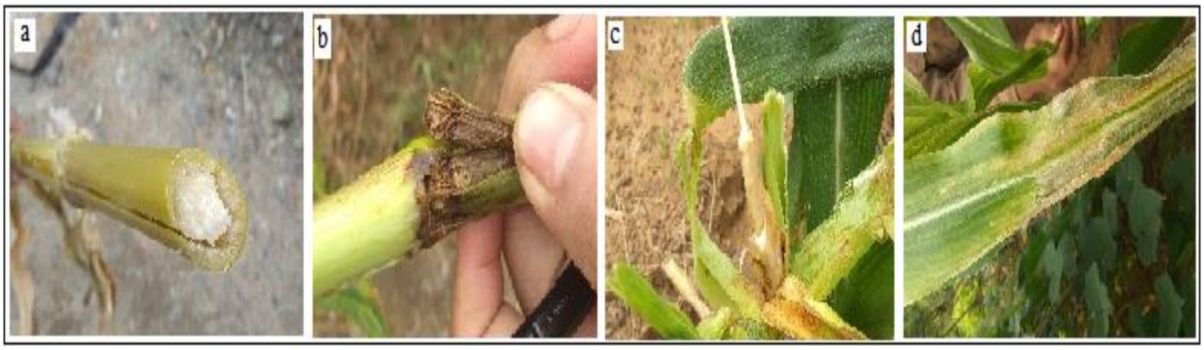

Fig. 1. Observation of symptoms in the field (Note: a. Symptoms of rot on the upper stalk; b. Symptoms of rot on the lower stalk; c. Symptoms of rot on plant shoots; d. Symptoms of blight on leaves).

After that, PCR amplification was performed on pure DNA samples (Fig. 5a). Isolates TL45 and J518 belonging to the genus *Pantoea* were tested with specific primer pairs for *Pantoea stewartii*, ES16 and ESIG2c, which were designed from the 16S-23S rDNA/ITS region. The sequence between the small subunit (16S) and the wide subunit (23S), or called RIS (Ribosomal Intergenic Spacer), is different for each species so that this sequence can be used as a marker for each particular species. Electrophoresis results from DNA amplification showed that the DNA band of the sample J518 was positive for *Pantoea stewartii* at 920 bp. However, the DNA was vaguely possible due to the small amount of DNA (Fig. 5b). Based on the results of this test, it was suspected that isolate J518 was *Pantoea stewartii.* It is consistent with the results of biochemical tests as described in Table 5.

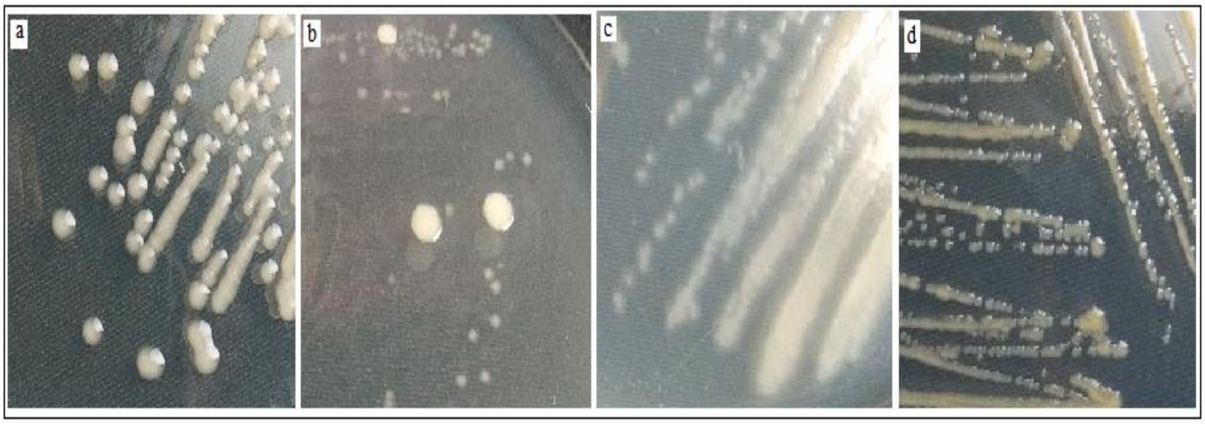

Fig. 2. Results of Isolation and Colony Observation (Note: a. Milky white bacterial colonies; b. Creamy bacterial colonies; c. Yellowish white bacterial colonies; d. Yellow bacterial colonies).

Discussion

Maize soft rot disease symptoms found in the field were rotting at the stalk base and upper stalk and leaf blight. Another symptom is slimy rot on young plants. Symptoms of wilt accompanied by rot can be caused by *Pantoea* sp., *Dickeya* sp. and *Pectobacterium* sp. (Pataky, 2003; Valenzuela *et al.*, 2007; de Haan *et al.*, 2008). *Pantoea stewartii* and *P. ananatis* are bacteria that cause bacterial leaf blight on shallots (Asrul *et al.*, 2019).

The symptoms shown by this bacterium are rotting at the seed stems and bulbs (Gitaitis and Gay 1997). Suryani (2012) found that *Pantoea stewartii*, which causes Stewart wilt disease in maize, can cause symptoms on the stalk if infected systemically.

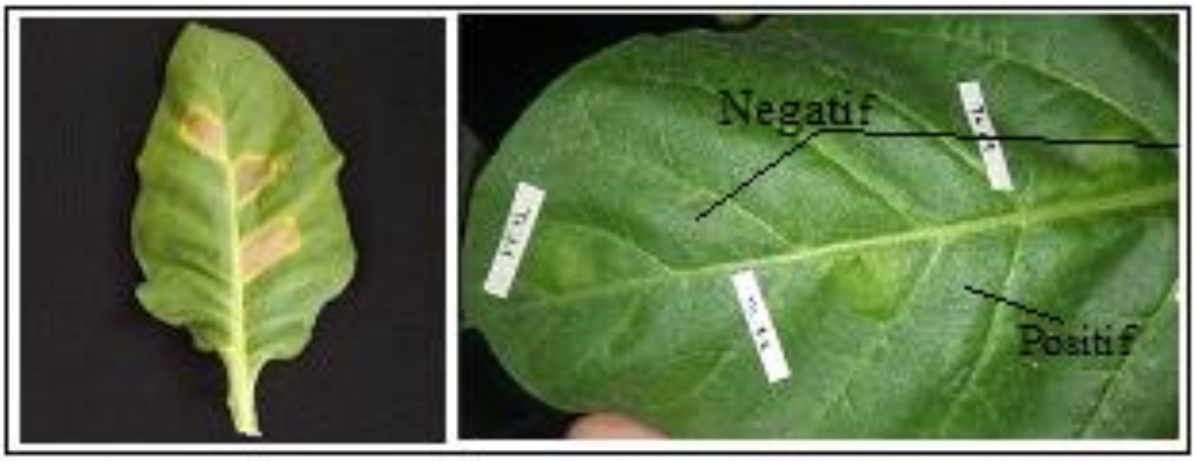

Fig. 3. Results of hypersensitivity test.

Recent research by Doblas-Ibáñez *et al.* (2019) has indicated that a dominant, heritable mechanism of resistance to the vascular spread of *P. stewartii* subsp. *stewartii* in maize seedlings involves the accumulation of electron-dense materials in the xylem, which impedes bacterial colonization of the vessels. They also demonstrated that the effector WtsE was essential for the dissemination of *P. stewartii* subsp. *stewartii* in the xylem.

Another bacterium found was *Pectobacterium*. This bacterium causes soft rot in many types of plants. The bacterium *P. carotovorum* subsp. *carotovorum* can cause blackleg disease in potatoes, characterized by a blackish discoloration at the base of the stem due to decay (Haan *et al.*, 2008). When viewed from the results of biochemical tests, the data obtained is relatively small. However, based on Rafiei *et al.* (2015) from several tests carried out on these 5 isolates, all of them had some similarities with *Pectobacterium brasiliense*. This bacterium causes soft rot disease in various types of vegetables. *Pectobacterium* (formerly *Erwinia*) *carotovorum* is a Gram-negative plant-specific pathogen, causing soft rot disease of various plant hosts and blackleg in potato by degradation of the plant cell wall (Aizawa, 2014).

Two isolates that were positive for the lecithinase test and included in the category of *Dickeya* sp. were isolate from Bajuin Village, Bajuin District and isolate from Ujung Baru Village, Bati Bati District. Based on the existing symptoms, these two isolates caused symptoms that matched the disease caused by bacteria of the genus *Dickeya* sp., namely soft rot on the stalk (Samson *et al.*, 2005). Symptoms of *Dickeya zeae* attack on corn: abnormal colours, necrotic area and wilting on the leaves; cortex with lesions on the roots, dieback, discoloration of bark, distortion, internal red necrosis, ooze and stunting or resetting on the stems CABI 2021. Symptoms of the disease included leaf wilting, the collapse of pseudostems, and unusual odor (Jingxin, 2014).

Dickeya sp. (*Erwinia chrysanthemi*) is reported to attack pineapple fruit, causing pineapple fruit rot

(Prasetyo and Aeny, 2014). According to Aeny *et al.* (2020), the species capable of hydrolyzing casein were 75% of the *D. Chrysanthemi* bv. *parthemi*, 80% of strain *D. dieffenbachiae*, and 75% of strain *D. dianticola*. Symptoms of damage to corn plants are stalk rot symptoms usually appear in midseason. The first and/or second internode above the soil line appears water-soaked, turns tan to brown, and becomes soft and mushy. Affected plants suddenly collapse and fall over with their vascular strands still intact. Infected tissue has a foul odor. In the top rot phase, tips of uppermost leaves wilt, followed by a slimy soft rot at the base of the whorl that spreads down into the stalk. Affected plants collapse (Pacific Northwest. Pest Management Handbook. Accessed on December 9, 2021).

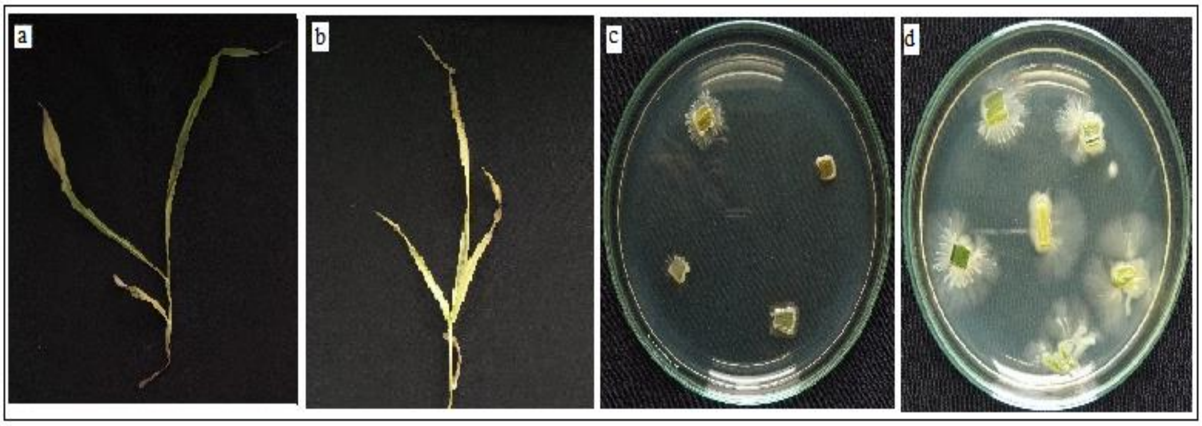

Fig. 4. Pathogenicity results (Note: a. Positive; b. Negative) and The results of Koch's postulates (Note: c. Positive; d. Negative).

Furthermore, PCR amplification was carried out on pure DNA samples. Isolates isolate from Bajuin Village, Bajuin District and isolate from Ujung Baru Village, Bati Bati District belonging to the genus *Pantoea* were tested with specific primer pairs for *Pantoea stewartii*, ES16 and ESIG2c, which were designed from the 16S-23S rDNA/ITS region (Coplin *et al.* 2002).

The sequence between the small subunit (16S) and the wide subunit (23S), or called the RIS (Ribosomal Intergenic Spacer), is different for each species so that this sequence can be used as a marker for each particular species (Yu and Mohn 2001). Electrophoresis results from DNA amplification showed that the DNA band from sample from Galam Sumsum Village, Bajuin District was positive for *Pantoea stewartii* at 920 bp. However, the DNA was vaguely possible due to the small amount of DNA. Based on the results of this test, it was suspected that isolate Galam Sumsum Village, Bajuin District was *Pantoea stewartii*. It is consistent with the results of biochemical tests.

Based on the manual by Schaad (2000), when viewed from the characteristics, isolates from Plasma Pulau Sari, Tambang Ulang District have a tendency to approach the species *Pantoea ananatis*. It is possible that these bacteria attack young corn plants and produce symptoms in the form of rotting of the stems and bases of young plants. These bacteria can attack several types of plants, such as pineapple, onions, corn, rice, tomatoes, and several other types of plants. Symptoms vary depending on the type of host infected, ranging from leaf spots, die-back, to rotting on stems, fruits, and tubers (Coutinho and Venter 2009). *P. ananatis* can act as an epiphyte as it has been isolated from asymptomatic rice and maize plants (Watanabe *et al.*, 1996; Paccola-Meirelles *et al.*, 2001). In addition, *P. ananatis* can also be endophytic. Under these conditions, they can be beneficial, neutral, or latent pathogenic (Lodewyckx *et al.*, 2002).

Isolate from Galam Sumsum Village, Bajuin District had similar characteristics to *Pantoea stewartii*. In the sample from Galam Sumsum Village, Bajuin

District, the symptoms indicated were not clear and there was only rotting of the scion of corn plants. Observation of symptoms cannot be done because the samples taken are old plants that have passed the harvest period so that the plants are dry enough.

The isolate with code J518 isolated from the sample from Galam Sumsum Village, Bajuin District was probably *P. stewartii* bacteria. *P. stewartii* bacteria is known as the cause of stewartii wilt in corn plants. The attack is characterized by the appearance of elongated spots parallel to the yellowish leaf bones and can cause rotting of the stems if attacked systemically (Suryani, 2012; Temaja *et al.*, 2018). *P stewartii* is known to only attack corn plants and several types of grasses that are still included in the corn family, namely *Euchlaena perennis* and *E. mexicana* (Elliot and Poos 1940).

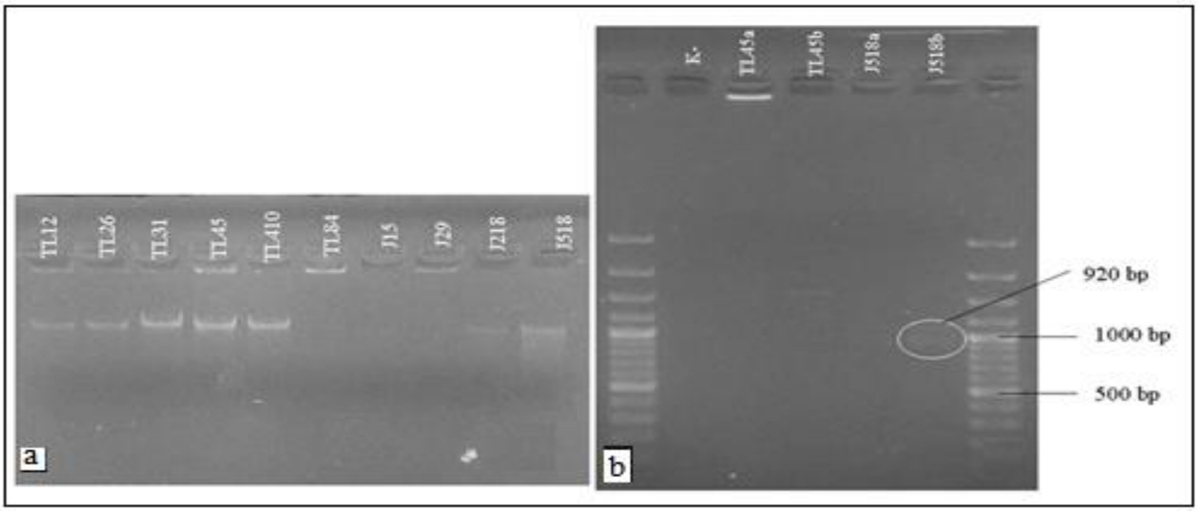

Fig. 5. Electrophoresis results from pure DNA (a) and PCR results of *Pantoea stewartia* (b).

Based on the identification test of the genus isolates from Tajau Pecah Village, Batu Ampar District, isolate from Plasma Pulau Sari, Tambang Ulang District and isolate from Tajau Pecah Village, Batu Ampar District categorized into the genus Pectobacterium bacteria. This bacterium is the cause of soft rot disease in various horticultural crops. Pectobacterium bacteria are divided into several species and subspecies. There are five different species of the genus Pectobacterium, namely *P. atrosepticum*, *P. betavaculorum*, *P. carotovorum*, *P. wasabiae* and *P. brasiliense* (Gardan *et al.*, 2003; Daurte *et al.*, 2004; Van der Merwe *et al.*, 2010). From several tests carried out on these 5 isolates, all of them had some similarities with *Pectobacterium brasiliense* which is a bacterium that causes soft rot disease in various types of vegetables. The main hosts of *P. brasiliense* are plants from the Solanaceae family. Five plants belonging to this family were reported to be infected by *P. brasiliense*, including potatoes, tomatoes, peppers, eggplant and tobacco. In addition, these bacteria also attack other plants such as cabbage, cucumbers, and other plants. Symptoms caused by *P. braasiliense* attack are soft rot of tubers, leaves and stems, necrosis, water soaking until brownish lesions appear on rootstock (Oulghazi *et al.*, 2021). Bacteria from the genera *Dickeya* and *Pectobacterium* also cause blackleg, stem rot and slow wilt in potato plants (van der Wolf. 2016).

Two isolates that were positive for the lecithinase test and included in the Dickeya category, namely isolate from Bajuin Village, Bajuin District and from Tajau Pecah Village, Batu Ampar District, based on the existing symptoms, these two isolates caused symptoms that matched the disease caused by bacteria of the Dickeya genus. The bacteria of the Dickeya genus known to attack maize are *D. zeae*, *D. dadantii*, and *D. paradisiaca*. Other hosts of this bacterium are potatoes, tomatoes, pineapples, bananas, and other plants (Samson *et al.*, 2005). Symptoms of this bacterial attack are generally wilting plants, rotting of the stems followed by blackening of the rotten parts (black leg) and rotting of tubers in bulbous plants such as potatoes (Ansermet *et al.*, 2016). The test using tartrate by Aeny *et al.* (2020) is divided into two types, namely D-tartrate and M-tartrate where the results are only different in *D. Chrysanthemi* bv. *parthemi* which 75% of the strains were able to use D-tartrate and 25% of the strains were able to use M-tartrate.

Conclusion

From the research conducted, it could be concluded that the bacteria that attack maize plants in the Tanah Laut Regency were bacteria belonging to the genus *Pantoea*, *Erwinia*, *Dickeya*, and *Pectobacterium*. Based on the biochemical test, the isolate from Plasma Pulau Sari, Tambang Ulang District was suspected to be *Pantoea anaatis*, and isolate Galam Sumsum Village, Bajuin District was suspected to be *Pantoea stewartii*. This result followed the results of PCR identification. Further biochemical testing is required for isolates belonging to the *Dickeya* and *Pectobacterium* genera. It is necessary to do a sequencing test to ensure that the isolate J518 is *Pantoea stewartii*. A PCR test with specific primers or different PCR cycles is required to confirm isolates suspected of being *Dickeya* spp.

References

Aeny TN, Suharjo R, Ginting C, Hapsoro D, Niswati A. 2020. Characterization and host range assessment of *Dickeya zeae* associated with pineapple soft rot disease in East Lampung, Indonesia. Biodiversitas **21(2),** 587-595.

Aizawa SI. 2014. *Pectobacterium carotovorum*-Subpolar Hyper-Flagellation. The Flagellar World. p 58–59.

Ansermet M, Shaerer S, Kellenberger I, Tallant M, Dupuis B. 2016. Influence of seed-borne and soil-carried inocula of *Dickeya* spp. on potato plant transpiration and symptom expression. European Journal of Plant Pathology **145(2),** 459-467.
http://doi.10.1007/s10658-016-0859-0

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. 1996. Current protocols in molecular biology. John Wiley and Sons, New York.

BBPOPT. 2015. Mengenal Penyakit Layu Stewart (Stewart Wilt) Pada Tanaman Jagung [Knowing Stewart Wilt Disease (Stewart Wilt) In Corn Plant]. https://bbpopt.tanamanpangan.pertanian.go.id/index.php/2015/12/30/stewart/

CABI. 2021. *Dickeya zeae* (Bacterial stalk rot of maize) Invasive Species Compendium. CABI. https://www.cabi.org/isc/datasheet/21944#toidentity.

Coplin DL, Majerczak DR, Zhang Y, Kim WS, Jock S, Geider K. 2002. Identification of Pantoea *stewartii* subsp. *stewartii* by PCR and strain differentiation by PFGE. PMID:30818612
https://doi.org/10.1094/PDIS.2002.86.3.304

Coutinho TA, Venter NS. 2009. *Pantoea ananatis*: an unconventional plant pathogen. Molecular Plant Pathology **10(3),** 325-335.
http://doi.10.1111/j.1364-3703.2009.00542.x

de Haan E, Dekker-Nooren T, Bovenkamp G, Speksnijder A, Zouwen P, Wolf J. 2008. *Pectobacterium carotovorum* subsp. *carotovorum* can cause potato blackleg in temperate climates. European Journal of Plant Pathology **122(4)**, 561-569.
http://doi.10.1007/s10658-008-9325-y

Doblas-Ibáñez P, Deng K, Vasquez MF, Giese L, Cobine PA, Kolkman JM, King H, Jamann TM, Balint-Kurti P , De La Fuente L, Nelson LJ, Mackey D, Smith LG. 2019. Dominant, heritable resistance to Stewart's wilt in maize is associated with an enhanced vascular defense response to infection with Pantoea stewartii. Molecular Plant-Microbe Interaction **32(12),** 1581–1597.
https://doi.org/10.1094/MPMI-05-19-0129-R

Duarte V, De Boer SH, Ward LL, de Oliveria AM. 2004. Characterization of a typical *Erwinia carotovora* strains causing blackleg of potato in

Brazil. Journal Applied Microbiology **96(3)**, 535-545. http://doi.10.1111/j.1365-2672.2004.02173.x.

Elliot C, Poos FW. 1940. Seasonal Development, Insect Vectors, and Host Range of Bacterial Wilt of Sweet Corn. Journal of Agricultural Research **60(10),** 645-686.

EPPO. (European and Medeterranean Plant Protection Organization). 2006. Pantoea stewartii subsp. stewartii. PM 7/60(1) OEPP/EPPO Bulletin **36,** 111–115.
www.eppo.org.

Gardan L, Gouy C, Christen R, Samson R. 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. International Journal of Systematic and Evolutionary Microbiology **53(2),** 381-391.
https://doi.org/10.1099/ijs.0.02423-0

Gitaitis RD, Gay JD. 1997. First Report of a Leaf Blight, Seed Stalk Rot, and Bulb Decay of Onion by *Pantoea ananas* in Georgia. Plant Disease **81(9),** 1096.
https://doi.org/10.1094/PDIS.1997.81.9.1096C.

Goszczynska T, Botha WJ, Venter SN, Coutinho TA. 2007. Isolation and Identification of the Causal Agent of Brown Stalk Rot, A New Disease of Maize in South Africa. Plant Disease 91, 711–718. PMID: 30780480
https://doi.org/10.1094/PDIS-91-6-0711

Jingxin Z, Shen H, Pu X, Lin B. 2014. Identification of *Dickeya zeae* as a Causal Agent of Bacterial Soft Rot in Banana in China. Plant Disease **98(4),** 436 – 447. PDIS-07-13-0711-RE.
http://dx.doi.org/10.1094 /

Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi, Mezgeay M, Van der Lelie D. 2002. Endophytic bacteria andtheir potential applications. Critical Reviews in Plant Sciences **21(6),** 583–606.
https://doi.org/10.1080/0735-260291044377

Neergaard P. 1977. Seed Pathology **1**, New York: John Wiley & Sons.

Oulghazi S, Sarfraz S, Zaczek-Moczydlowska MA, Khayi S, Ed-Dra A, Lekbach Y, Faure D. 2021. *Pectobacterium brasiliense*: Genomics, Host Range and Disease Management Microorganisms **9(1),** 106.
https://doi.org/10.3390/microorganisms9010106

Paccola-Meirelles LD, Ferreira AS, Meirelles WF, Marriel IE, Casela CR. 2001. Detection of a bacterium associated with leafspot disease of maize in Brazil. Journal of Phytopathology **149(5),** 275–279.
http://doi.10.1046/j.1439-0434.2001.00614.x.

Pacific Northwest. Pest Management Handbook. Printed page URL:
https://pnwhandbooks.org/plantdisease/hostdisease / corn-zea-mays-bacterial-stalk-rot.
https://pnwhandbooks.org/node/2542

Pataky JK. 2003. *Stewart's Wilt of Corn. In* The American Phytopathological Society: apsnet.org

Prasetyo J, Aeny TN. 2014. Pineapple Fruit Collapse: Newly emerging disease of pineapple fruit in Lampung, Indonesia. Jurnal Hama dan Penyakit Tumbuhan Tropika **14(1),** 96–99.
https://doi.org/10.23960/j.hptt.11496-99

Purnawati F. 2019. Pentingnya Jagung Sebagai Komoditi Ekspor dan Impor di Indonesia [The Importance of Corn as an Export and Import Commodity in Indonesia]. Class II Agricultural Quarantine Center Yogyakarta: https: yogya.karantina.pertanian.go.id.

Rafiei S, Khodakaramian G, Baghaee-Ravari S. 2015. Characterization of *Pectobacterium* Species Isolated from Vegetable Crops in North-West of Iran. African Journal of Agricultural Research **10(46),** 4258-4267.
http://doi.org/10.5897/AJAR2015.9551

Roper MC. 2011. *Pantoea stewartii* subsp. *stewartii*: Lessons learned from a xylem-dwelling pathogen of sweet corn. Molecular Plant Pathology **12(7),** 628–637. https://doi.org/10.1111/j.1364-3703.2010.00698.x

Samson R, Legendre JB, Christen R, Saux MFL, Achouak W, Gardan L. 2005. Transfer of *Pectobacterium chrysanthemi* and *Brenneria paradisiaca* to the Genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and Delineation of Four Novel Species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. International Journal of Systematic and Evolutionary Microbiologi **55(4),** 415-1427. https://doi.org/10.1099/ijs.0.02791-0

Schaad N, Jones J, Chun W. 2000. *Laboratory Guide for Identification of Plant Pathogenic Bacteria, Third Edition.* St Paul. American Phytopathological Society Press.

Shurtleff MC. 1980. Compendium of Corn Diseases. Second Edition. APS Press. The American Psychopathological Society.

Sudjono MS. 2018. Penyakit jagung dan pengendaliannya [Corn Disease and Its Control]. Cereal Crops Research Institute. https://www.balitsereal.litbang.pertanian.go.id.

Suryani L, Aini LQ, Sugiharto AN, Abadi AL. 2012. Characterization of Bacterial Pathogen Causing Wilt and Leaf Blight on Corn *zea mays*) by Physiological, Biochemical and Molecular Methods. Agrivita **34(3),** 286–295. http://doi.org/10.17503/agrivita.v34i3.169

Valenzuela GM, Rojas HS, Martinez DO, Moctezuma EV, Zuniga BA, Molina LZ, Quezada GA. 2007. First Report of *Pantoea agglomerans* Causing Leaf Blight and elete Vascular Wilt in Maize and Sorghum in Mexico. Plant Disease **91(10),** 1365. http://doi.10.1094/PDIS-91-10-1365A.

Van der Merwe JJ, Coutinho TA, Korsten L, Van deer Waals E. 2010. *Pectobacterium carotovorum* subsp. *brasiliensis* causing blackleg of potatoes in South Africa. European Journal of Plant Pathology **126(2),** 175-185. http://doi.10.1007/s10658-009-9531-2

van der Wolf JM, de Haan EG, Kastelein P, Krijger M, de Haas BH, Velvis H, Mendes O, Kooman-Gersmann M, van der Zouwen PS. 2016. Virulence of *Pectobacterium carotovorum* subsp. *brasiliense* on potato compared with that of other *Pectobacterium* and *Dickeya* species under climatic conditions prevailing in the Netherlands. Plant Pathology **66(4),** 571 – 583. https://doi.org/10.1111/ppa.12600.

Watanabe K, Kawakita H, Sato M. 1996. Epiphytic bacterium, *Erwinia ananas*, commonly isolated from rice plants and brown plan-thoppers (*Nilaparvata lugens*) in hopperburn patches. Applied Entomology and Zoology **31(3),** 459-462. https://doi.org/10.1303/aez.31.459

Yu Z, Mohn WW. 2001. Bacterial Diversity and Community Structure in An Aerated Lagoon Revealed by Ribosomal DNA Sequencing. Applied and Environmental Microbiology **67(4),** 1565-1574. http://doi.org/10.1128/AEM.67.4.1565-1574.2001