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Inventory and identification of banana cultivars and diseases caused by bacterial and fungal pathogens in West Timor, East Nusa Tenggara Province, Indonesia

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Abstract. *Henuk JBD, Kadja DH, Mau YS. 2020. Inventory and identification of banana cultivars and diseases caused by bacterial and fungal pathogens in West Timor, East Nusa Tenggara Province, Indonesia. Trop Drylands 4: 10-16.* Banana is one of the most important horticulture crops in West Timor, East Nusa Tenggara Province, Indonesia. But the productivity of this crop is still low due to many factors, one of which is diseases caused by bacterial and fungal pathogens. There are many banana cultivars in West Timor but information on their cultivar identity and the diseases they suffer are absent. The present study aimed at: (i) identifying the banana cultivars in West Timor, (ii) assessing the symptoms of the diseases on the banana cultivars, and (iii) identifying the pathogens causing the diseases. The sampling of research location was done purposively while sampling of banana plantation was using a snowball method. Banana cultivar showing bacterial blood and fungal disease symptoms were further examined to isolate and to identify the pathogens based on the pathogen morphological characteristics. Twenty-one banana cultivars were found in West Timor, and were included in three genomic groups, i.e., ABB, AAB, and AA. Symptoms and signs of bacterial infection were only found on Pisang Kepok. Two bacteria isolates and soil samples were further cultured on specific and general bacterial media and also subjected to gram staining, all of which revealed that the bacterial isolates were not the causal bacterial pathogens of Blood disease or banana *Xanthomonas* wilt. Thus, no blood disease or banana *Xanthomonas* wilt is present in West Timor at the moment. Various and abundant fungal disease symptoms were exhibited by most of the cultivars. Banana diseases caused by fungal pathogens included Cordana Spot disease (*Cordana musae*), Anthracnose disease (*Colletotrichum musae*), Sigatoka disease (*Mychosphaerella* sp.), Leaf spot disease (*Phyllachora musicola*), *Curvularia* spot disease (*Curvularia* sp.) and Freckle disease (*Phyllosticta musarum*).

Keywords: Inventory, identification, bacterial and fungal pathogens, banana cultivar, West Timor

INTRODUCTION

A number of bacterial diseases of banana had been reported to occur in Indonesia. The diseases included blood disease (blood disease bacterium/BDB) caused by *Pseudomonas celebensis*, moko disease caused by *Ralstonia solanacearum* race 2, and bacterial wilt caused by *Xanthomonas vasicola* pv. *musacearum* (Semangun 2007). Banana diseases caused by bacteria have also been reported to occur explosively during the last few years in Sumba Island, East Nusa Tenggara (ENT) Province, Indonesia. The local government of Southwest Sumba District, ENT Province reported that banana plantations in the region had been infected by *Xanthomonas* wilt disease with very high intensity, especially on the local cultivars called Pisang Kapok/Pisang Marmia and Pisang Ambon, which caused total loss of the crop. Our preliminary study (Mau and Henuk 2016) also revealed disease symptoms typical to those caused by *X. vasicola* pv. *musacearum*, indicated by rotten flowers and premature ripened fruits, and yellow bacterial ooze inside the premature fruits. *Xanthomonas* wilt pathogen attacks the banana plant very quickly and even can cause plant death in just one month (Mackie et al. 2007). Therefore, it is recommended that the infected plants have to be eradicated as soon as possible to reduce losses caused by the disease. In addition to bacterial diseases, fungal diseases of banana have been endemic in this region. The information on the kind of diseases in banana and their symptoms is lacking despite the highly abundant banana cultivars that exist in ENT Province (Mudita 2012).

The banana disease pathogens are easily transmitted through water, wind, soil, vectors such as insects and birds, contaminated harvesting equipment, infected planting materials, etc. These may have facilitated the dangerous bacterial diseases such as banana blood disease to be spread from Sumba Island to the neighboring Islands in ENT Province such as Timor Island, Flores Island, etc. The uncontrolled transportation of banana products and its planting materials among islands in ENT Province may have caused the banana disease to spread along with the islands.

Timor Island is a major Island in ENT Province as well as a banana producing center in the province. To prevent the banana diseases epidemic in this island, especially the bacteria-causing diseases such as that happened in Sumba

Island, it is necessary to do an inventory and identification of the diseases caused by bacterial pathogens, especially those of vascular bacterial pathogens attacking banana plantations in Timor Island. In addition to bacterial diseases, banana diseases caused by fungal pathogens may cause considerable losses as well, depending on the banana cultivars. There are many banana cultivars that exist in West Timor but information on their cultivar identity and the diseases they suffer are not available at present. The objectives of this study were to carry out an inventory of banana diseases caused by bacterial and fungal pathogens and to identify the causal pathogens. The study results will be used as a basis for formulating the development strategy of bananas in ENT Province, and West Timor Island in particular, as well as the development of disease management strategy through, among others, the use of banana cultivars resistant to important diseases of bananas in the region.

MATERIALS AND METHODS

Study area and period

This study was conducted in banana plantations growing districts in West Timor, Indonesia, i.e., Kupang District, Timor Tengah Selatan (TTS) District, and Malaka District from May to October 2016. A survey method was used in this study focusing on the villages of banana production centers. The villages were purposively selected based on information on the number of farmers having banana plantations. A multi-stage snowball sampling method was used to determine the villages (study location) and farmer households having banana plantations. In the selected village, the first farmer household owning banana plantation was selected, and the next farmer household was determined based on information obtained from the first farmer household, and so forth until a total of three farmer households were met in each selected village. In each district, a total of 15 farmer households having banana plantations were interviewed and their banana plants were observed for the various diseases.

Identification of banana cultivars

Selected farmers were interviewed for the name (local name) and type of banana cultivars they grow in their fields, followed by direct observation of the banana cultivars for identification of morphological characters. Identification of banana cultivars in the field was based on banana genome notation proposed by Simmonds and Shepherd (1955). Differentiation of banana cultivars for genomic notation was based on 15 morphological characters of the banana (Mudita 2012). Pictures of the morphological characters were taken and then used to determine the banana genome. Each morphological character was given a score of unity (1) if it showed the character of *Musa acuminata* and a score of five (5) if it showed the character of *M. balbisiana*. Banana cultivars that showed morphological characters in between the two species were given scores of 2, 3, or 4, depending on the level of their similarities with the two species. Those with morphological characters more closely resembling *M. acuminata* were scored 2, and those in between the two were scored 3, and those more resembling *M. balbisiana* were scored 4. The *M. acuminata* banana genome was denoted by the letter A and the *M. balbisiana* banana genome with the letter B. Scores obtained from the 15 characters were then summed and used to determine the banana cultivar genome as shown in Table 1.

Sampling of banana plants for disease symptoms assessment

Disease symptom assessment was done separately for each banana cultivar. A total of three healthy and three diseased plants were observed for each banana cultivar. The sampled plants were determined using a stratified systematic sampling method. Assessment of disease signs and symptoms were carried out with the assistance of pictorial field key/guide of various banana diseases.

Identification of the diseases and pathogens

Infected parts of diseased banana plants were then taken to laboratory for further identification of the causal pathogens by using a conventional technique. Laboratory preparations included the sterilization of equipment by using an autoclave at 121˚C for 15-20 minutes; and preparation of the growing media to isolate pathogens that are thought to be pathogenic bacteria in vascular tissue and the fungal pathogens in the infected leaf tissue.

The Nutrient Agar (NA) medium was used to isolate the pathogenic bacteria. The prepared selective medium for *Xanthomonas* pathogenic bacteria was a Yeast Dextrose Carbonate Agar (YDCA) medium, for *Ralstonia* was a Tetrazolium Chloride (TZC) medium and for *Pseudomonas* was a King's B medium.

Table 1. Examples of banana genomic groups based on 15 morphological characters (Mudita 2012)

Genomic group	Score	Examples of local banana cultivar
AA/AAA	15-25	*Musa* AA 'Pisang Mas', *Musa* AAA 'Pisang Ambon Hijau', *Musa* AAA 'Pisang Susu'
AAB	26-46	*Musa* AAB 'Pisang Raja', *Musa* AAB 'Pisang Raja Sereh', *Musa* AAB 'Pisang Tanduk'
AB/AABB	47-49	-
ABB	59-63	*Musa* ABB 'Pisang Awak'
ABBB	67-69	-
BB/BBB	70-75	*Musa* BBB 'Pisang Kepok'

Infected parts of pseudostem were cut into small pieces and immediately inserted into a test tube containing 5 ml of sterile distilled water. Ooze of the bacteria from the stem pieces in the distilled water was allowed to stand for a few minutes to obtain sufficient amount of suspension for culturing the bacteria in the media. The bacteria were cultured by scraping a loop of bacterial suspension onto the media in a petri dish using a quadrant method. Each growing bacterium was then further sub-cultured to obtain a pure culture, which then further subjected to gram staining or KOH assay.

Each type of pathogenic bacterium that causes disease in banana plant samples was identified by using a conventional technique. Conventional identification of bacteria was done using guidelines of Pests and Disease Image Library (PaDIL, http://www.padil.gov.au) and Plant Pathology Guidelines for Master Gardeners (http://erec.ifas.ufl.edu/plant_pathology_guidelines/module_05.shtml). Fungal pathogens were identified using guidelines of Pests and Disease Image Library (PaDIL, http://www.padil.gov.au), MycoBank (http://www.mycobank.org/Biolomics.aspx?Table=Mycobank&Page=200&ViewMode=Basic), and APSNet (http://www.apsnet.org/edcenter/Pages/phi.aspx#illustrated glossary). Disease symptoms and signs were observed in the infected banana plants, and their diseases symptoms, were presented in forms of photographs.

Isolation and identification of fungal pathogens are described as the following. Sample of each disease symptom was surface sterilized by dipping it into sodium hypochlorite (2%) solution for 30 seconds followed by dipping in 70% ethanol for one minute, then was rinsed twice using sterile distilled water. Infected tissue of approximately 2 mm x 2 mm size was cut from the edge of the symptom or lesion, then was planted in a petri dish containing half-strength medium of Potato Dextrose Agar (1/2 PDA) [19 g PDA + 10 g Agar in 1 L distilled water) provided with antibiotics (0.2 g L^{-1} novobiocin) to inhibit bacterial growth. The petri dish was then incubated for 3-7 days at room temperature (25 °C). A pure culture was then made from each growing colony.

Microscopic characters of the fungal pathogen were observed with a light microscope. Macroscopic observations were made on the color of the colony, the shape of the colony, and the time or duration of growth of the pathogen.

RESULTS AND DISCUSSION

Banana cultivars found in West Timor

Study results revealed that 11 banana cultivars (local name) were grown by farmers in Kupang District i.e., Pisang Kepok, Pisang Rote, Pisang Ambon, Pisang Beranga, Pisang Susu, Pisang Raja, Pisang Tembaga Merah, Pisang Tembaga Putih, Pisang Meja, Pisang Kulit Mentah, and Pisang Amerika. Meanwhile, 15 banana cultivars were found to be grown by farmers in Timor Tengah Selatan (TTS) District; nine of which were similar to those grown by farmers in Kupang District (Pisang Kepok, Pisang Rote, Pisang Ambon, Pisang Susu, Pisang Cavendish, Pisang Meja, Pisang Tembaga Merah, Pisang Kulit Mentah, Pisang Amerika), and the rest were Pisang Mas, Pisang Luan, Pisang Aceh, Pisang Singapura, Pisang Dilli, and Pisang Pinang. In Malaka District, 11 banana cultivars were found, i.e., Pisang Kepok, Pisang Ambon, Pisang Meja, Pisang Susu, Pisang Raja, Pisang Luan, Pisang Batu, Pisang Kulit Mentah, Pisang Kafendis, Pisang Asam, and Pisang Mas.

Interview results revealed that each farmer usually cultivates 3-5 cultivars of banana around their home yard and gardens. Banana cultivars commonly found in the fields were Pisang Kepok, Pisang Rote, Pisang Susu, Pisang Tembaga, Pisang Meja, Pisang Ambon, and Pisang Kulit Mentah. These types of bananas are scattered throughout the observation area in the districts of Kupang, TTS, and Malaka, both in the highlands and the lowlands, as these banana cultivars are very easy to grow on various soil conditions and are drought resistant. These banana cultivars have been well-known by the community in the research areas.

Almost all parts of banana were utilized by the respondents. Pseudo stems and leaves of banana are usually used as fodder (for cattle and pigs). Besides, the leaves are also commonly used for packaging various types of food and cakes. The banana flowers commonly called the male buds are used for public consumption as vegetables, while the ripened fruits are consumed directly, or used as materials for a wide variety of cakes and snacks and even as an alternative food.

Bananas have an important role in food security for local communities. FAO (2009) reported that the banana is a staple food in many developing countries, including Uganda, Burundi, and Rwanda, because people in these countries consume 3-11 bananas per day or 250-400 kg per year (Biodiversity 2012). In West Timor, unripe fruit of Pisang Luan cultivar is boiled or steamed and consumed as an alternative food (Mudita 2012).

Banana cultivar identification

Based on the 15 morphological characters descriptors used to identify and distinguish the cultivars of banana by genome notation proposed by Simmonds and Shepherd (1955), the banana cultivars in the three districts in West Timor are presented in Table 2. Table 2 shows that there are three banana genome groups in West Timor, namely genomic groups ABB, AAB, and AA. The cultivars included in the ABB genomic group were Pisang Kepok, Pisang Rote, Pisang Singapura, Pisang Amerika, Pisang Pinang, Pisang Aceh, and those in the AAB genomic group are Pisang Ambon, Pisang, Pisang Cavendish, Pisang Raja, Pisang Asam, and Pisang Dilli. The AA genomic group consisted of Pisang Susu, Pisang Tembaga, Pisang Tembaga Merah, Pisang Tembaga Putih, Pisang Meja, Pisang Kulit Mentah, Pisang Baranga, Pisang Mas, Pisang Luan, and Pisang Batu.

Table 2. List of Banana Cultivar's Genome found in West Timor, Indonesia

Local name of banana	Score*	Genomic group**
Pisang Kepok	65	ABB
Pisang Rote	67	ABB
Pisang Ambon	34	AAB
Pisang Susu	20	AA
Pisang Kafendis	26	AAB
Pisang Tembaga	24	AA
Pisang Tembaga merah	24	AA
Pisang Tembaga putih	19	AA
Pisang Meja	16	AA
Pisang Kulit mentah	20	AA
Pisang Amerika	67	ABB
Pisang Baranga	19	AA
Pisang Raja	26	AAB
Pisang Mas	19	AA
Pisang Luan	19	AA
Pisang Asam	26	AAB
Pisang Singapura	65	ABB
Pisang Dilli	28	AAB
Pisang Pinang	59	ABB
Pisang Aceh	65	ABB
Pisang Batu	20	AA

Note: *Score was based on 15 morphological characters. ** Genomic group was based on value ranges score of the 15 morphological characters

Symptoms of banana diseases

The results showed that the signs and symptoms of banana diseases caused by bacterial pathogens were very rarely found. There were only a few plants of Pisang Kepok that were found to exhibit symptoms and signs of diseases caused by bacterial pathogens, i.e., in Merbaun Village of Kupang District (Figure 1.A), in the villages of Oebobo, Mio, and Batu Putih of TTS District (Figures 1.B and 1.C).

Signs and symptoms of diseases allegedly caused by pathogenic bacteria were used to perform visual identification as well as macroscopic and microscopic examinations. Visually, disease symptoms found on Pisang Kepok cultivar in three villages were thought to be caused by bacterial pathogens, indicated by all the leaves that turned yellow, necrotic, wilted, and hanging down due to broken and even the tree was fallen. The ooze is the main characteristic of pathogenic bacteria infection, however, there was no bacterial ooze produced/found when the pseudostem was cut horizontally, and instead, the middle part of the pseudostem was destroyed and produced a very foul smelling. The fruits of the infected plant turned to mummy but again, no bacterial ooze was observed when the fruits were cut into pieces. The pseudostem of banana plant from Mio Village, TTS District also shared the same symptoms of that from Kupang District, however, when the pseudostem was cut transversely, there was a hole along the middle section of the pseudostem without rotten smell, but there were larvae of insect at the end of the hole. In a normal condition, the pseudostem, including the middle section, is in a compact form that contains fiber that comprises polymers such as cellulose, hemicellulose, pectin, and lignin (Manilal and Sony 2011).

External symptoms that appeared on the leaves were almost the same as the description of the symptoms of blood diseases by CABI (2013), i.e., if the pathogenic bacteria of blood disease infect a tissue vessel and the fruit, it will cause yellowing of all the leaves with gradual necrosis, then withered, broken and hanging down. However, internal symptoms of pseudostem and fruit of diseased banana plants in this study produced no signs or symptoms of pathogenic bacteria infection as there was no bacterial ooze found. According to Promusa (2013), internal symptoms and signs in pseudostem are yellow mucus that comes out of the vessel when the stem is cut. The same symptoms and signs can also be seen in other parts of the infected tree. Based on descriptions of symptoms and signs of CABI (2013) and Promusa (2013), external and internal symptoms found on Pisang Kepok cultivars was not caused by blood disease and *Xanthomonas* wilt bacteria, but to ensure these allegations, the plant tissue and soil samples were isolated and grown in NA medium, and then was purified in YDCA as a specific medium for *Xanthomonas*, and in TZC as a specific medium for *Ralstonia*. If the bacteria could grow on the TZC and YDCA media, their morphological characteristics were identified macroscopically and microscopically by conventional techniques.

Plant and soil samples that were isolated and grown in NA medium produced two different isolates (Figures 2 and 3). Isolates 1 had a cloudy white colony color, shiny, round shape, slightly convex, shiny, and translucent, while isolates 2 had a round form flat-edged colonies, bright yellow color or striking, somewhat translucent, convex and shiny. Gram staining was carried out to determine the gram characteristic of both bacterial isolates. Gram staining revealed that both bacterial isolates could not grow on YDCA and TZC media, but they grew normally in an NA media. Thus, these two bacterial isolates were not identified further because it is certain that both bacterial isolates are not the pathogens of blood disease or banana *Xanthomonas* wilt; and they are most likely secondary bacterial present in the infected banana plant.

In contrast to the limited number of bacteria causing banana disease signs and symptoms, there were abundant signs and symptoms of banana diseases caused by fungal pathogens in the research area. Various banana cultivars, included Pisang Kepok, were found to exhibit fungal disease signs and symptoms as presented in Table 1. Signs and symptoms of each of these diseases are typical and easily characterized by visual inspection. Visual identification of the disease was assisted by various pictorial images of each of the diseases, and the causal pathogen was identified through microscopic characters of each pathogen (Table 1).

Table 1 shows that various cultivars of banana in the research areas, including Pisang Kepok, suffered various diseases caused by fungal pathogens; the diseases included Cordana Spot disease, Anthracnose disease, Sigatoka disease, Leaf spot disease, *Curvularia* spot disease, and Freckle disease.

Figure 1. Symptoms and signs of bacterial pathogens on Pisang Kepok cultivar in Kupang and TTS Districts. A. in Merbaun Village of Merbaun, Kupang District, B. in Oebobo Village, TTS District, C. in Mio Village, TTS District

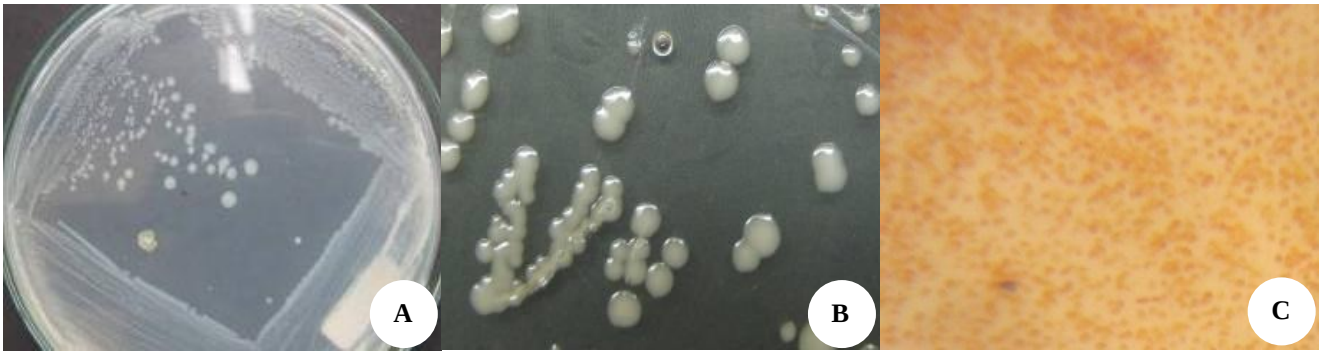


Figure 2. Growth characteristics of bacteria isolated from soil samples on NA Medium: A. Bacterial growth on the petridish, B. Bacterial colony, C, Gram staining

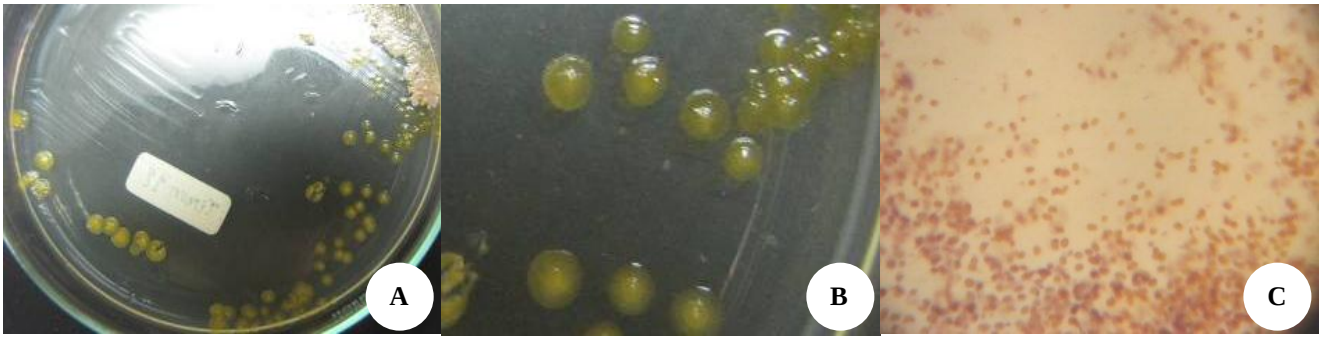
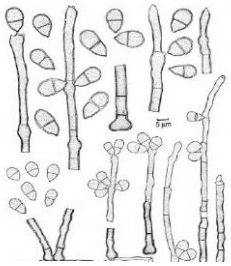
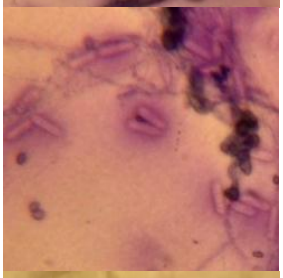
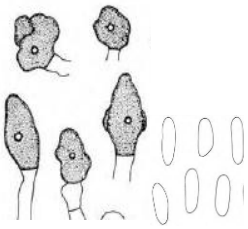
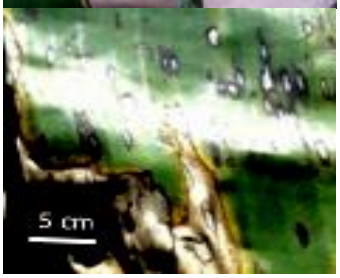
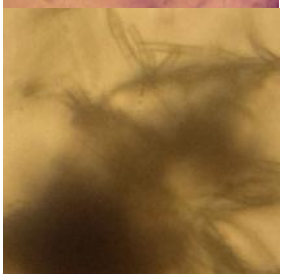
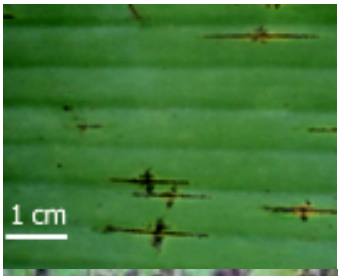
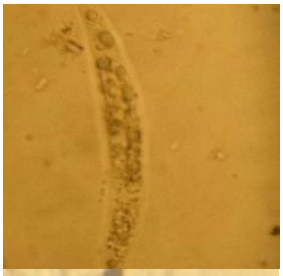
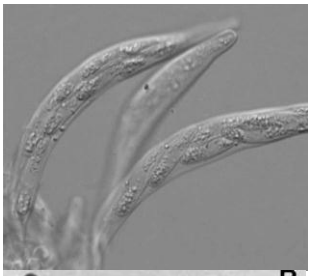
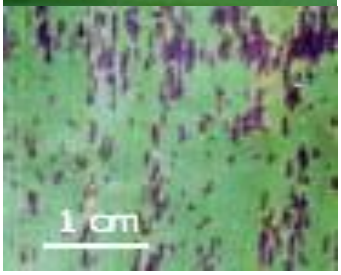
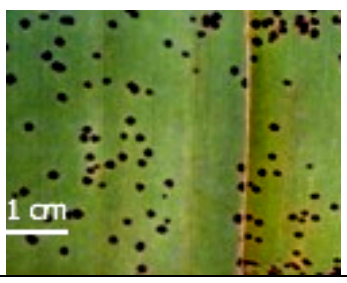


Figure 3. Growth characteristics of bacterial isolates from infected plant samples on NA Medium: A. Bacterial growth on the Petri dish, B. Bacterial colony, C. Gram staining

Table 1. Various symptoms and signs of observed banana diseases, observed and reference microscopic characters of pathogens causing the diseases, and description of microscopic characters of the pathogens. A: Cordana Spot disease (*Cordana musae*), B: Anthracnose disease (*Colletotrichum musae*), C: Sigatoka disease (*Mychosphaerella* sp.), D: Leaf spot-cross bar disease (*Phyllachora musicola*), E: *Curvularia* spot disease (*Curvularia* sp.), F: Freckle disease (*Phyllosticta musarum*)

	Disease symptom	Observed microscopic character of the pathogen	Reference picture for the microscopic character of the pathogen	Description of the microscopic character of the pathogen
A				Cordana Spot disease is caused by *Cordana musae*. *C. musae* has a stick-shaped conidiophore and pyriform conidia. In the middle of the conidia, there is a black septum that divides the conidia into two cells (Ploetz 2003).
B				Anthracnose disease is caused *by Colletotrichum musae*, which has brown appressoria. The appressoria are formed from hyphae, single conidia, hyaline, and elliptical shape (Ploetz 2003).
C				The causal pathogen of Sigatoka disease is *Mychosphaerella* sp., which has a stroma; pale brown, insulated and branched conidiophores; the conidia are sub-hyaline, cylindrical and generally consist of 5 septa (Liberato et al. 2011).
D				Leaf spot-cross bar disease, is caused by *Phyllachora musicola*. *P. musicola* has a clavate-shaped, sharp edge, hyaline and non-insulated ascospores (Liberato 2006).
E				The causal pathogen of *Curvularia* spot disease is *Curvularia* sp. *Curvullaria* sp. has an insulated, oval, and curved or one-sided like curve conidia. The conidia are formed in groups at the end of the conidiophores. One group of conidia usually consists of at least 3 cells (Watanabe 2002).
F				Freckle disease is caused by *Phyllosticta musarum*, which has a group of round to oval conidia shapes (Liberato et al. 2011).

To conclude, there are 21 banana cultivars that have been cultivated by farmers in West Timor, ENT Province, Indonesia. The banana cultivars can be classified into three genomic groups, i.e., ABB, AAB, and AA. Banana cultivars included in ABB group were Pisang Kepok, Pisang Rote, Pisang Singapura, Pisang Amerika, Pisang Pinang, and Pisang Aceh, and those in the AAB genomic group are Pisang Ambon, Pisang Kafendis, Pisang Raja, Pisang Asam, and Pisang Dilli. The AA genomic group consisted of Pisang Susu, Pisang Tembaga, Pisang Tembaga Merah, Pisang Tembaga Putih, Pisang Meja, Pisang Kulit Mentah, Pisang Baranga, Pisang Mas, Pisang Luan, and Pisang Batu. Signs and symptoms of diseases caused by pathogenic bacteria were only found on Pisang Kepok cultivar in Kupang and TTS Districts but the bacteria were not the causal pathogens of Blood Disease or banana *Xanthomonas* wilt; they are most likely secondary bacterial present in the infected banana plant. Banana diseases caused by fungal pathogens included Cordana Spot disease (*Cordana musae*), Anthracnose disease (*Colletotrichum musae*), Sigatoka disease (*Mychosphaerella* sp.), Leaf spot disease (*Phyllachora musicola*), *Curvularia* spot disease (*Curvularia* sp.) and Freckle disease (*Phyllosticta musarum*).

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