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Status and Recent Developments of *Pantoea stewartii* subsp. *stewartii* Causes the Wilt Disease in Maize in Indonesia: A Review

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Abstract

Pantoea stewartii subsp. *stewartii* is a pathogen that causes Stewart wilt on maize. This disease is important in maize. Infection early in growth can cause yield losses of up to 100%. This review examines the history, recent developments, economic impacts and developments of detection technology of Stewart wilt diseases. This paper was created by reviewing several articles relevant to the purpose of the topic. The article's results revealed that the pathogen underwent many changes, including its name, taxonomy, physiological abilities, status as an important pathogen in many countries and the development of detection technology. Currently, *P. stewartii* subsp. *stewartii* belongs to the Erwiniaceae family and has physiological abilities that can be distinguished from bacteria of the same genus and species. This pathogen has been reported to spread to more than 82 countries, including Indonesia, with 18 host plants. Some areas have reported pathogens in Indonesia, but no vector has been written. The biggest economic threat caused by this disease is the industrial production of corn seeds which require strict phytosanitary requirements and are free from pathogens. To anticipate its spread, there are four methods of detection of pathogenic bacteria that are commonly used, but serological and molecular detection technologies are the main recommendations.

Keywords: bacterial wilt of maize; Erwiniaceae; phytopathogen; serological and molecular detection; Stewart wilt

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INTRODUCTION

Pantoea stewartii subsp. *stewartii* is a pathogenic bacterium that causes Stewart wilt in maize. Stewart wilt is spread through seeds and insect vectors. Commonly by *Chaetocnema pulicaria,* where the bacteria can survive throughout the winter and then enter the host tissue through wounds when insects eat (Stewart, 1898; Mergaert et al., 1993; Pataky and Ikin, 2003; Cushatt, 2020). The survival ability of these bacteria is supported by the presence of special proteins that play a major role in the adaptation of *Pantoea* spp. to various conditions (De Maayer et al., 2012).

P. stewartii subsp. *stewartii* was first reported by Stewart (1898) as a bacterium that causes wilt disease in sweet corn on Long Island, New York. This disease originates from the Queens area of New York, infecting sweet corn (Manhattan) plants. In the same experiment, Stewart also reported that infected sweet corn

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plants at the beginning of the growth phase would end in death, but at this time, this pathogen did not yet have a name.

Stewart wilt disease originates and is endemic in North America. Many have been reported to infect maize plants, especially sweet corn, in several producing countries (Stewart, 1898; Pataky and Ikin, 2003; EPPO, 2022a). This disease is also very important because it can cause yield losses of up to 100%, especially in susceptible hosts and infected seeds (Pataky and Ikin, 2003). Some countries even impose restrictions on the import of seeds to prevent the possible entry of pathogens (Pal et al., 2019). The spread of these bacteria as dangerous organisms to enter and spread in the European Union is regulated by the European Commission (2000). In Indonesia, the rules regarding the prohibition of the entry of pathogens are stated in the regulation of the Ministry of Agriculture regarding the types of quarantine plant-disturbing organisms.

Wilt disease in maize seriously threatens sustainable food production and ecosystems worldwide (Strange and Scott, 2005; Bragard et al., 2019). Disease management is currently mostly done using synthetic materials. The use of synthetic materials to produce two important sectors, namely the environment and the economy of business actors (Tanumihardjo et al., 2019). In terms of management, the most important point is prevention with early detection of the presence of pathogens, so prevention of limitation of distribution areas is the wisest management method. This management can begin with the early detection of the presence of the disease by understanding the symptoms and signs (Liu and Wang, 2021). Understanding this disease is important because there are many strains of *P. stewartii* with many similarities in physiological abilities and host ranges (Ren et al., 2020; Agarwal et al., 2021). Accuracy of detection and identification is the key to effective prevention of spread which means there is a danger of Stewart wilt disease and large yield losses if infection occurs at the beginning of planting and in susceptible varieties (generally sweet corn).

Plants infected with this pathogen will cause characteristic symptoms. The plant leaves will experience blight and necrosis with a development pattern in the direction of the leaf bone with a slightly wavy leaf shape. The boundaries between healthy leaves and dead tissue are very clear (different from other blight symptoms). Symptoms that develop will cause necrosis of all parts of the leaf to all parts of the plant. Early infection (seed transmission) in plants is susceptible to direct death without causing early symptoms in the leaves (Rice and Munkvold, 2000; Djaenuddin and Muis, 2018).

Currently, the spread of this disease is reported not only in the states of North America. Still, it has also spread to several large continents such as South America, Africa, Europe and Asia (EPPO, 2022a). In Indonesia, Stewart wilt disease of corn is known to have spread in the archipelago such as East Java, West Sumatra, West Java and Bali (Suryani et al., 2012; Rahma et al., 2013, 2014; Temaja et al., 2017) and other large islands such as Sulawesi and Lombok-West Southeast Nusa (Ministry of Agriculture, 2020). The application of detection technology and knowledge is also important for technical implementation in the field to detect the entry and endemic of this disease.

Technology for detecting plant diseases caused by bacterial groups is mostly done using pathogens' morphological and physiological detection methods. Along with the rapid development of technology, the application of detection and development globally has grown (Lamka et al., 1990; Nechwatal et al., 2018; Pal et al., 2019). This development was initiated by several developed countries, especially countries that have an interest in the export and import of corn seeds (Pataky and Ikin, 2003; Jeger et al., 2018). Various management efforts have been carried out intensively, one of which is applying pest status for *P. stewartii* subsp. *stewartii* is supported by several detection technology developments to prevent the spread, especially in national and global trade. Among these technologies, serological and molecular detection have recently undergone many developments. One of the most recent changes is a detection system with a nanoparticle-based biosensor to increase detection speed and detection. This approach was initially used to detect human pathogens, which can be adopted to apply to plant pathogens (Dyussembayev et al., 2021).

Generally, the Gram-negative group of pathogenic bacteria use the type III secretion system (T3SS) to initiate infection. The type III secretion system uses effector proteins to suppress and initiate disease progression (Galán and

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Collmer, 1999). The control function of two transcription factors, Lrp and IscR, is important for *P. stewartii*. These two transcription factors affect the colonization process, growth in maize and their effect on host growth (Bartholomew et al., 2022). De Maayer et al. (2011) reported that the pantoea genus also involves VgrG and Hcp proteins which indirectly involve the type IV secretion system. Merighi et al. (2005) demonstrated that HRpS-mediated autoregulation is caused by hrpS activation by increased HRpY levels resulting from hrpXY transcription of the hrpL promoter. Autoregulatory loops can rapidly induce hrp genes during infection, compensate for negative regulatory mechanisms and maintain regulation in insect vectors.

This knowledge provides an illustration that knowing and knowing *P. stewartii* subsp. *stewartii* carried out through PCR amplification is effective if it is carried out using specific primers encoded based on genes involved in the virulence process, such as cps and hrp virulence genes. The presence of this gene also revealed the presence of virulence in the pathogen carrying out pathogenicity in jackfruit (Ibrahim et al., 2019).

Development of information related to *P. stewartii* subsp. *stewartii* is still developing in line with the development of pathogen detection/identification technology. Recent information reports on the development of pathogens in the distribution area and host plants other than maize. The development of this knowledge is important so that it can be compiled as a source of current information for all people in understanding Stewart's wilt disease. This review examines information about the history, recent developments, economic impacts and developments of detection technology of Stewart wilt diseases.

MATERIALS AND METHOD

This review paper was created by reviewing several relevant articles to determine the status and current developments of *P. stewartii* subsp. *stewartii* is the cause of Stewart wilt in corn in Indonesia. For this purpose, authors reviewed several journal articles, proceedings, conferences, books, sections of books, dissertations, theses and online sites on the status, recent developments, distribution, impact and recommendations of the management of Stewart wilt on maize. Once the data were collected, the authors summarized all the information. At the end of the discussion, some important information was written to serve as additional knowledge, reference and can be used as recommendations for managing wilt disease in maize or other crops that become pathogenic.

RESULTS AND DISCUSSION

History, development of taxonomy and characteristics

Since it was discovered as a pathogenic bacterium that causes lay on corn plants, this bacterium has had many names that have changed along with the development of knowledge about its shape, morphology, physiology and molecular composition. When this bacterium was discovered, it was named *Pseudomonas stewartii*. Then the name was changed to *Bacterium stewartii*. Then it changes back to *Aplanobacter stewartii* based on its morphology which does not have a flagellum. Furthermore, the name of the bacteria was again changed to *Bacillus stewartii*, then *Phytomonas stewartii* (Smith), then *Xhanthomonas stewartii* based on the similarity of morphological features with the genus *Xhanthomonas*. Then in 1962 based on the similarity of its physical properties. Pathogenic bacteria changed their name to *Erwinia stewartii* (Smith) (Lamka, 1990; Block, 1996; Cushatt, 2020). Some of these name changes were made based on the morphological and physiological characteristics of the pathogen. Further, Mergaert et al. (1993) then proposed that this pathogen is included into the genus Pantoea with the name *P. stewartii* based on the examination of the results of the soluble protein electropherogram. This experiment also proved the differences between species and subspecies in the proposed genus Pantoea, namely *P. ananas*, *P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes* based on differences in their physiological abilities. Specifically, some research strengthened the results obtained from these studies. Identification using polymerase chain reaction technology with its various innovations confirms genomic differences in strains within the *P. stewartii* species (Thapa et al., 2012; Li et al., 2019; Rahimi-Khameneh et al., 2019). This naming is maintained until now and is widely known. Recently, the genus Pantoea, formerly a family of Enterobacteriaceae, has become a family of Erwiniaceae with the genus Pantoea (Brady

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et al., 2010; Roper, 2011; Adeolu et al., 2016). In detail, the taxonomy of *P. stewartii* is divided into Kingdom: Bacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Enterobacteriales, Family: Erwiniaceae, Genus: Pantoea (EPPO, 2022a).

There is a lot of information on pathogens, including gram-negative, rod-shaped bacteria without flagella and facultative anaerobes. Yellow pigment color, mucoid form without pores, size 0.4 to 0.8 x 0.9 to 2.2 m. Some strains are known to grow at temperatures of at least 4 °C and a maximum of 37 °C. *P. stewartii* is also known to produce extracellular polysaccharides, protein WceF, quorum sensing signaling and acyl-homoserine lactone (AHL), which is related to its pathogenicity (Duong et al., 2017; Irmscher et al., 2021).

Status and impact of the geographical distribution of *P. stewartii* subsp. *stewartii*

Since it was identified as a cause of wilt in sweet corn on New York's Long Island, *P. stewartii* began to be reported to damage crops in other areas of the United States such as New Jersey, Iowa, mid-Atlantic, Ohio River Valley region, in the southern Corn Belt, Arkansas, Delaware, Illinois, Indiana, Kentucky, Maryland, Missouri, Ohio, Pennsylvania, Tennessee, Virginia and West Virginia (Rand and Cash, 1937; Cushatt, 2020). This spread started an epidemic of *P. stewartii* in that country which then led to endemic status in most countries in North America. EPPO (2022a) reported the distribution of *P. stewartii* subsp. *stewartii* in 82 countries in the world (Figure 1). This distribution does not include Indonesia, so it can be ascertained that this pathogen has spread to more than 82 countries.

In Indonesia, this bacterium was formerly a pathogen of Quarantine Plant Pest Organism (QPPO) A1 (plant destruction organisms that do not yet exist and should not be entered) based on regulations issued by the Ministry of Agriculture. Rahma et al. (2014), first reported wilting symptoms in corn plants in West Pasaman Regency, West Sumatra, Indonesia. Subsequent reports in East Java Province revealed blight accompanied by wilt in sweet corn plants in Batu Regency in 2011. After isolation, characterization and molecular testing, information was obtained that the disease was caused by the bacterium *P. stewartii* subsp. *stewartii* (Suryani et al., 2012). Information regarding the presence of pathogens in Indonesia has also been reported in several other areas such as Java, Sumatra, Bali, Sulawesi and Lombok (Rahma et al., 2014; Temaja et al., 2017; Ministry of Agriculture, 2020). Thus, only Kalimantan and Papua are the islands in the archipelago, where this disease has not been reported. It automatically changes the pathogen status from QPPO A1 to QPPO A2 (already present in certain areas and still prohibits its wider spread).

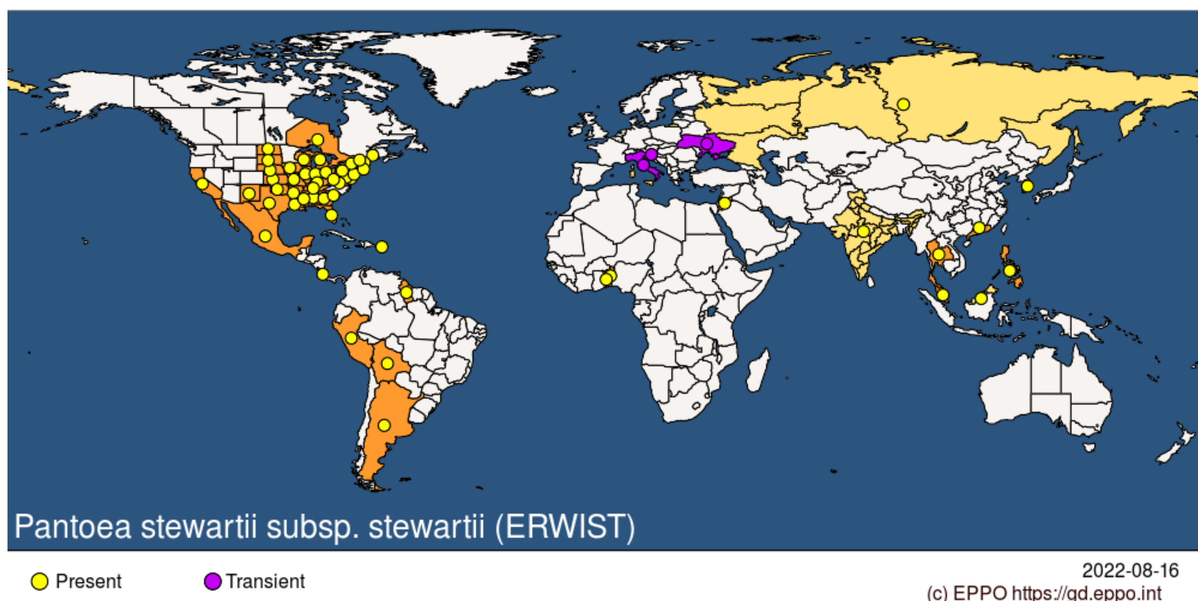

Figure 1. Current distribution of Stewart wilt caused by *P. stewartii* subsp. *stewartii* (EPPO, 2022b)

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In addition to its widespread, Stewart's wilt seriously impacts the world economy. In the United States, this disease causes considerable losses to the sweet corn and seed industry (Bragard et al., 2019). These losses arise as a result of the sustainable use of sweet corn hybrid seeds known to be susceptible but planted in areas where there are vectors of the disease. Yield loss in sweet corn is influenced by the level of resistance, the time of occurrence of pathogen infection and the presence of insect vectors in the planting area (Pataky and Ikin, 2003). In general, sweet corn production was not significantly affected if the resistant hybrid varieties were cultivated. Yield loss is less than 10% but can reach 90% if susceptible hybrid maize is cultivated, especially if infection occurs at the beginning of the planting phase (Pataky and Ikin, 2003).

Currently, the corn seed industry is the biggest threat posed by Stewart wilt. Corn seed production in some endemic areas requires strict phytosanitary requirements for maize seed production for local use. It requires seed production areas to be free from pathogens for export seed. Especially if the seeds are produced from the United States, more than 100 countries worldwide apply quarantine regulations on imports of corn seeds from the area (Pal et al., 2019). Phytosanitary restrictions imposed by trading partners have an impact on the seed trade. Not only limiting the export of seeds but also incurring additional costs for phytosanitary examinations and laboratory tests. Seed producers also incur indirect costs from developing Stewart wilt-resistant maize varieties. Treatment with insecticides is also another additional cost. The use of certified seeds is an effective way to reduce the spread of disease. Leaf insecticides are also an additional input if planting is in an endemic area (Cushatt, 2020). This risk is also a national threat in Indonesia, considering that Indonesia is one of the producers and consumers of corn, both food corn and sweet corn, which are currently popular.

Distribution of *P. stewartii* subsp. *stewartii* to host plants

Early findings suggested that Stewart wilt in sweet corn was initiated by using infected plant seeds and planting in infected soil. However, in recent years research results have shown seeds as a source of disease inoculum in the field (Rand and Cash, 1937; Djaenuddin and Muis, 2018; Pal et al., 2019). Research on seeds as a source of inoculum in the field has been carried out with the result that less than 2% of diseased plants originated from infected plant seeds (Michener et al., 2002). These results concluded that the rate of disease transmission through seeds is very low. This information is evidence that vectors have a major role in spreading and conserving pathogens in nature. However, it is known that the spread of disease between countries and continents is more on movement through trade in source seeds (Bragard et al., 2019).

Several research results revealed that *P. stewartii* subsp. *stewartii* is transmitted by insect vectors that feed on diseased plants, which then move to feed on healthy plants (Ammar et al., 2014). Several insects are suspected of being disease vectors; *C. pulicaria*, *Agrotis manchus*, *C. denticulata*, *Diabrotica nigricornis*, *D. undecimpuctata*, *D. versifera*, *Hylemya cilicrura* (Ministry of Agriculture, 2020). In fact, there is minimal information regarding several insects as vectors of Stewart wilt disease other than *C. pulicaria* and *C. denticulata*. More specifically, it stated that *C. pulicaria* is the only species important for the long-term survival of bacteria during the winter. In Indonesia, Widodo et al. (2017) have even confirmed that insects of the same genus, namely *C. basalis* fed to maize, were infected with *P. stewartii* subsp. *stewartii* is not capable of transmitting the disease to healthy maize plants. The same experiment also confirmed that even the Stewart wilt vector had not been found in Indonesia, especially Sumatra and Java. Their absence in the field, and the absence of reports regarding the presence of Stewart disease vectors in corn plants is strong evidence that these two insects have not yet entered Indonesia. So, it is strongly suspected that the pathogen entered Indonesia due to the seed trade.

Host range, symptoms and mechanisms of *P. stewartii* subsp. *stewartii* infection in host plants

P. stewartii subsp. *stewartii* has four main hosts: *Zea mays* (maize) and *Zea mays* subsp. *mays* (sweet corn), *Zea mays* subsp. *exadece* (teosinte) and *Zea mays* subsp. *parviglumis*, and 14 alternative hosts, including *Agrostis gigantea*, *Artocarpus heterophyllus*, *Coix lacryma-jobi* L., *Dactylis glomerata*, *Digitaria*, *Dracaena sanderiana*, *Oryza sativa*, *Panicum capillare*, *P. dichotomiflorum*, *Poa pratensis*, *Setaria lutescens*, *Sorghum sudanense*, *Tripsacum dactyloides* and *Triticum aestivum* (EPPO, 2022a) (Figure 2).

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Figure 2. Symptoms and Signs of *P. stewartii* subsp. *stewartii* infection on the host; A). Maize-leaf blight (EPPO, 2022b); B). Jackfruit-bronzing (Abidin et al., 2020); C). Necrosis-*D. sanderiana* (Choi and Kim, 2013)

Symptoms of disease due to infection with *P. stewartii* subsp. *stewartii* can be divided into two distinct phases: seed wilting and leaf blight. The wilting phase occurs when the plant is infected early in growth and is a susceptible variety (Cushatt, 2020). This infection usually originates from seed infection and is the basis for the development of artificial inoculation techniques to test pathogens' pathogenicity with several modifications, including injecting stems into young seedlings. Infection at the beginning of growth causes a faster death than infection when the plant is older, where the climatic conditions favor the development of infection. The process then ends with the death of the plant. In infection by insects (usually in endemic areas), initial symptoms are linear lesions wet with fluid from vector bites (Ammar et al., 2014). The bacteria then spread throughout the plant after multiplying and accumulating around the infected tissue. The lesions then expanded and caused all the leaves to wither and die. The plants will become stunted, seems white at the end of the season and eventually shrivel and die (Rice and Munkvold, 2000).

The second phase of Stewart wilt infection is the blight phase. This phase is the most common in the field and generally does not cause plant death. The leaf blight phase can occur in all phases of plant growth, but the symptoms are more pronounced in the generative phase. Early symptoms appear on the side where the disease vector eats the plant. The bacteria will multiply in the leaves and xylem, causing yellowish lesions and developing into necrotic streaks with wavy edges along the leaves. These lesions can extend the entire length of the leaf and cause the entire leaf to brown and die. It can also be limited to only a few centimeters from the side of infection, depending on the level of plant resistance. Severe cases of leaf blight can also cause leaf death and ultimately reduce crop yields (Pataky and Ikin, 2003).

Most of the transmission of Stewart wilt disease in maize occurs through an insect vector, the *C. pulicaria* corn flea beetle. These bacteria can survive the winter while in the intestines of vector insects. The vector transmits disease by feeding in the spring after hibernation during the winter (Correa et al., 2012). The first phase of the disease occurs in the apoplast and involves the type III secretion system with effectors causing drenching sores on the leaves. Early in the disease phase, bacteria migrate to the plant xylem, grow with high cell densities and form a biofilm that blocks water transport, causing wilting and death of young seedlings. The disease will be limited to late blight symptoms if more

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mature maize plants are infected. However, these infected plants become a source of inoculum for subsequent infection infestations (Roper, 2011; Doblas-Lbéfiez et al., 2019).

P. stewartii subsp. *stewartii* uses two type III secretion system located in separate parts of the mega-plasmid that have a role in infecting the host, one for maize and the other for vectors (Correa et al., 2012: Duong et al., 2017). *P. stewartii* subsp. *stewartii* can communicate quorum sensing between one individual to another. Quorum sensing *P. stewartii* subsp. *stewartii* is a function of the interaction between the AHL, produced by a LuxI homologue named EsaI and the transcription factor EsaR homologous LuxR at high cell densities. In contrast to the LuxI/R regulatory system, EsaR is a two-level transcription factor that suppresses or activates its target at low cell density and no interaction with AHL; EsaI inactivity occurs when the EsaR-AHL complex is formed. Quorum sensing plays an important role in the virulence of *P. stewartii* in maize (von Bodman et al., 1998).

This quorum sensing-mediated virulence is due to the regulation of the expression of Stewartan exopolysaccharide (EPS) *P. stewartii* subsp. *stewartii* and surface motility (von Bodman et al., 1998; Herrera et al., 2008). Two transcription factors directly controlled by EsaR, RcsA and LrhA, have been reported to play important roles in the regulation of EPS production and surface motility of *P. stewartii* subsp. *stewartii*, respectively (Duong et al., 2017). Another pathogenicity factor is *P. stewartii* subsp. *stewartii* in plant colonization Hrp (response hypersensitivity and pathogenicity) type III secretion system expresses effector proteins, such as WtsE, into plant host cells upon direct contact. Expression of this system occurs during plant growth under *in vitro* assay conditions (Packard et al., 2017; Doblas-Lbéfiez et al., 2019).

Detection and identification of bacteria *P. stewartii* subsp. *stewartii*

Detection and identification of pathogens is an important step in the integrated disease control process. The faster the detection and the more accurate the identification results, the faster and more accurate the recommendations for controlling the target pathogen will be. Regarding Stewart wilt disease caused by infection with the pathogen *P. stewartii* subsp. *stewartii*, detection and identification methods have been developed. This development occurs along with the advancement of technology and the increasing number of researchers who study it. Several detection methods can be grouped into four general methods, namely 1) visual detection and identification in the field, 2) *in vitro* morphological, physiological, and biochemical detection and identification, 3) serological detection and identification, 4) molecular detection and identification using polymerase chain reaction (PCR) machine amplified by electrophoresis machine. The most common detection method is visual detection method. This detection is done by looking at the symptoms caused by *P. stewartii* on its host plant. In general, plants look wilted and dry and leaf blight with a pattern of necrosis parallel to the leaf veins accompanied by a wavy leaf shape. Another symptom is the stunted size of the plant (Stewart, 1898). In addition, disease detection is also often accompanied by the presence of vectors in the field, signs of disease such as bacterial fluids (fluid) as well as symptoms of necrotic cavity tissue. Visually, detection resistant varieties can be seen from a significant decrease in production.

Detection and identification physiologically and biochemically *in vitro*, referring to Schaad et al. (2001) instructions for identification of bacteria *in vitro*. The process of determining the genus of Pantoea bacteria is characterized by several characteristics, including gram-negative, yellow colonies, rod shape, not flagellated, does not form spores, can grow under anaerobic conditions, cause hypersensitivity reactions in tobacco plants and can form yellow pigment on YDC and King'B media (Figure 3). Testing is carried out to determine the species level by looking at the specific abilities of bacteria in the same genus. *P. stewartii* subsp. *stewartii* has several specific characteristics, such as not producing indole; not hydrolyzing esculin; not grow on citrate, mucin, cis-aconitate and DL-4-aminobutyrate. The main cellular fatty acids are octadecenoic acid (C18:1), exadecenoic acid (C16:0) and cis-9-hexadecenoic acid (C16:1) (Mergaert et al., 1993), produced acetoin, gelatinase and catalase (Choi and Kim, 2013), able to infect the main host plant in Koch's postulate test and range. Morphological, physiological and biochemical detection methods are the most commonly used because they are considered more economically affordable. In addition, not all regions have molecular detection and identification facilities, including most areas in eastern Indonesia. However,

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in recent years, detection and identification have been carried out by combining physiological, biochemical and molecular testing methods for the detection and identification of the presence of pathogenic bacteria (Suryani et al., 2012; Choi and Kim, 2013; Temaja et al., 2017; Abidin et al., 2020).

One of the detection and identification techniques that are also carried out for pathogenic bacteria is the serological method, better known as ELISA (Enzym linked immunosorbent assay). Initially, this technique was only used to detect plant viruses in the 1970s. Currently, this method has been widely used to detect other pathogens, including bacteria. Lamka (1990) used the ELISA method to confirm *P. stewartii* subsp. *stewartii* from several isolated strains from America, Block (1996) used the ELISA method to detect the spread of pathogens through sweet corn seeds in the field and Michener et al. (2002) used the ELISA method to prove and calculate the percentage of disease spread through infected plant seeds. Feng et al. (2015) developed a detection method for *P. stewartii* subsp. *stewartii* used immunochromatographic strips, then with the same model, Zhang et al. (2014) added lanthanide as a label to increase light emission to improve the visualization of marking lines. Some of these studies prove that serological methods with several developments are perfect for detecting and identifying *P. stewartii* subsp. *stewartii*. This method is also a priority detection method along with molecular test methods to complement the results of physiological and biochemical testing of pathogens (EUPHRESCO, 2009; EPPO, 2016). The advantages of the serological test method are that it can be carried out with many samples at once, low cost, short testing time with high accuracy. This advantage makes serological tests the main choice for detecting the traffic spread of the disease through the seed trade. In Indonesia, restrictions on the traffic of pathogens are contained in Ministry of Agriculture (2020).

Along with the times, the modernization of pathogen detection technology is improving. The molecular detection method of *P. stewartii* subsp. *stewartii* is one of the newest with some of his method developments. PCR technology detects Pantoea bacteria growing in vitro on plant parts or seeds (Kini et al., 2021). This method generally uses specific primers to detect down to the species level (Gehring et al., 2014; Cui et al., 2021). Pal et al. (2019) used real-time PCR and conventional PCR detection methods to differentiate *P. stewartii* subsp. *stewartii* with *P. stewartii* subsp. *indologenes* on infected maize seeds. Tambong et al. (2015) used universal and specific primers in one reaction (multiplex PCR using 16S rRNA, leuS, gyrB, rpoB, cpsD) followed by hybridization of digoxigenin-labeled amplicons to 22 specific oligonucleotide probes (19- to 24-mers) immobilized on nylon membranes. Uematsu et al. (2015) using loop-mediated isothermal amplification (LAMP) technology for practical Stewart wilt disease surveillance in the field. Choi and Kim (2013) used PCR detection to see *P. stewartii* subsp. *stewartii* as the cause of wilt disease in bamboo fortune plants (*D. sanderiana*). De Maayer et al. (2017) performed secretion markers to differentiate between *P. stewartii* subsp. *stewartii* with other strains. Hernandez-Morales et al. (2017); Abidin et al. (2020) stated, perform molecular characterization and phylogenetic analysis-based PCR to detect *P. stewartii* subsp. *stewartii* causes bronzing disease in jackfruit in Mexico and Malaysia. This strain has been fully genome known and stored in Nucleotides Europe with accession number ERP119356 and study accession number PRJEB36196 (Ibrahim et al., 2019; 2020).

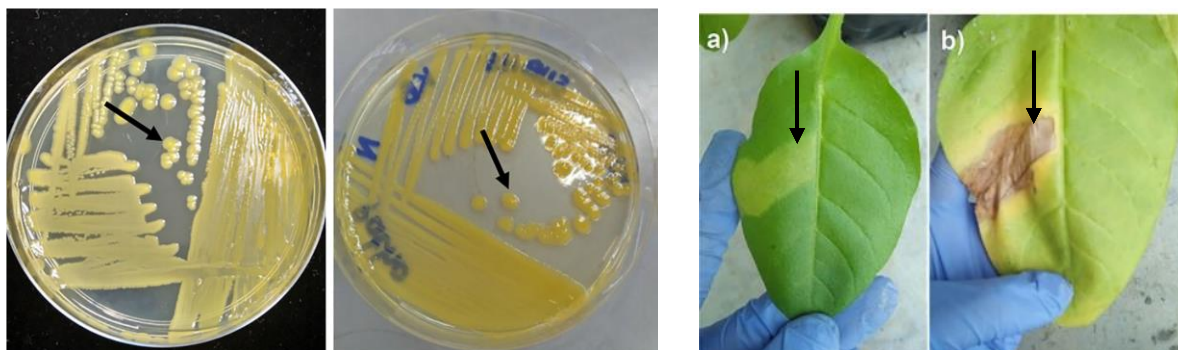

Figure 3. Bacterial colony morphology on King'B (left side) and hypersensitivity reactions tobacco plants 12 and 24 hours after infiltration (right side) (Abidin et al., 2020)

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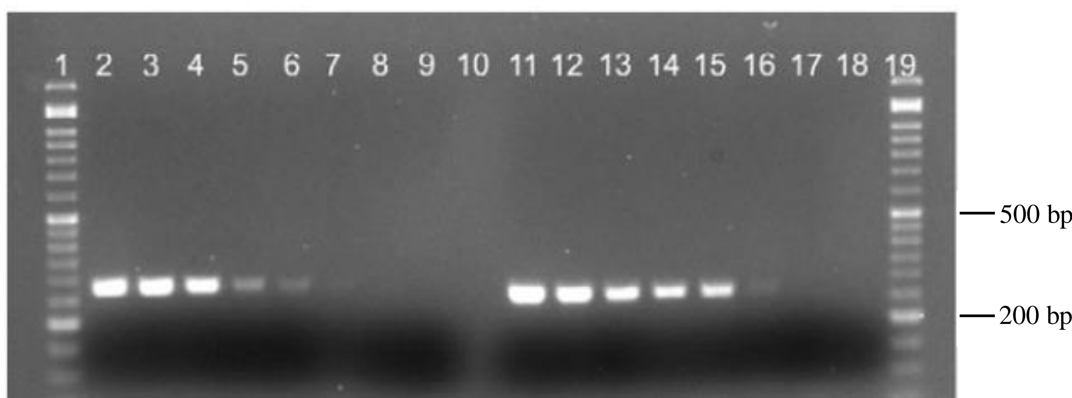

Figure 4. PCR products of DNA extracts of samples infected *P. stewartii* subsp. *stewartii*, amplified with primers DC283galE/ DC283galEc. PCR reactions without IPC primers Lanes 1 and 19, FastLoad 50 bp DNA Ladder (Serva); lanes 2–7, seed extracts spiked with 10^6, 10^5, 10^4, 10^3, 10^2 or 10^1 cells ml^{-1}, lane 8, NIC (negative internal control) seed extract; lane 17, NIC leaf extract; lanes 9 and 18, Empty; lane 10 (Nechwatal et al., 2018)

In Indonesia, several studies related to the detection and identification of *P. stewartii* subsp. *stewartii* using PCR has been widely used. Suryani et al. (2012); Rahma et al. (2014); Temaja et al. (2017) reported the early presence of pathogens in several areas in Indonesia using the molecular detection-PCR method, Desi et al. (2014) carried out the characteristics of isolates of *P. stewartii* subsp. *stewartii* on corn in West Sumatra also used PCR. Widodo et al. (2017) used PCR detection to detect *P. stewartii* subsp. *stewartii* in Java and Sumatra, as well as confirming the presence of *C. basalis* in Indonesia, is not a vector of pathogens. Globally and nationally, molecular detection methods are the main recommendations for detection, identification or confirmation of the development of new detection methods. Wensing et al. (2010) even used conventional PCR techniques to confirm the results of the development of the MALDI-TOF MS method, which detects and identifies differences between species in the Pantoea genus through mass spectrometry analysis of bacteria which are then differentiated in dendrogram analysis. Nechwatal et al. (2018) recommend this molecular method as the main screening in the detection of *P. stewartii* subsp. *stewartii* on seed and leaf tissue of plants (Figure 4). Molecular screening of *P. stewartii* subsp. *stewartii* has been developed with the innovation of using special primers to detect certain genes (Tambong et al., 2015; Zhang and Qiu, 2015; Shin et al., 2022).

The latest in detecting plant pathogens uses biosensor technology as a modern detection tool (Dyussembayev et al., 2021). This technology is widely used in environmental monitoring, the detection of pesticide residues and the real-time detection of pathogens in the world of human health (Liu et al., 2018). The biosensor is a device that integrates a biological sensing element and a physicochemical transducer to generate a specific electronic signal. The output of this technology is in the form of biomolecular interactions in the form of digital information. Elements that act as bioreceptors in the form of antibodies, DNA, enzymes, tissue types and parts of cells (Sawant, 2017; Elmer and White, 2018).

CONCLUSIONS

Stewart wilt is a significant disease of maize caused by the *P. stewartii* subsp. *stewartii*. This bacterium is an Erwiniaceae family previously included in the Enterobacteriaceae family. *P. stewartii* subsp. *stewartii* is a world quarantine pest that has been reported in 82 countries, including Indonesia, with a total of 18 host ranges. The global economic impact is a decrease in production, an increase in production costs and phytosanitary costs. Early prevention globally with the use of nano biosensing technology needs to be applied as a form of anticipation of a wider spread.

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