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The presence of bacterial stalk rot disease on corn in Indonesia: A review

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Abstract. Bacterial stalk rot disease in corn results in a significant reduction in yield due to the interruption of the flow of nutrients from the roots to other parts of the plant. Pathogenic bacteria infect the inner tissue of the stalk until it rots. This disease has been reported to attack corn crops in Asia and Europe such as India, Korea, Thailand, Philippines, Nepal, Mexico, Serbia, and China. In Indonesia, this disease was first reported to attack corn in the West Sulawesi region by the Mamuju Class II Quarantine Station. The results of molecular identification indicated that this disease is caused by the bacterium *Dickeya zea*, previously known as *Erwinia chrysanthemi* pv. *zea* that previously reported attacked pineapple and aloe vera in Indonesia. The potential for economic losses due to this disease is quite high, so appropriate and efficient control measures are needed. Based on those, this research study about the symptom, the characteristic of the bacteria agent caused the stalk rot disease, the distribution and the impact to the maize production in Indonesia.

1. Introduction

Corn (*Zea mays* L.) is a cereal plant that is widely cultivated by farmers in Indonesia to fulfill food and animal feed needs. Corn is currently a strategic national commodity and in the economic nomenclature of food crops in Indonesia, corn is the second important commodity after rice [1]. National corn production in 2019 reached 22.59 million tons with the main producing areas or centers of corn harvesting area in Indonesia distributed in ten provinces with a contribution of 83.53% to Indonesia's total harvested area [2]. The ten regions are East Java, Central Java, Lampung, North Sumatra, South Sulawesi, West Nusa Tenggara, West Java, North Sulawesi, Gorontalo and South Sumatra.

Corn harvested area for the 2015-2019 period continues to increase by an average of 4.38% per year [2]. This is of course supported by several factors including natural resources, agroecological environment, and government policies to support increased corn production. Currently, the increase in corn production continues to be driven through a Corporate Food Crop Area Development Program (ProPaktani) to increase production and exports so that the agricultural sector becomes stronger as a support for the national economy. Another program is the procurement of seeds through the Seed Independent Area Model under the coordination of IAARD and Seed Independent Village under the coordination of the Directorate General of Food Crops [1]. Hipi et al (2015) in [3] stated that fostering farmer groups in the Seed Independent Village program in several provinces succeeded in producing



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quality hybrid corn seeds. This certainly has an impact on surrounding farmers because it can reduce the cost of corn production in the form of lower seed prices because it is produced from the local area.

Efforts to increase corn production continue to be carried out to meet the growing demand for corn as a raw material for livestock. Corn is mostly chosen as a constituent of livestock feed because it is easy to digest, palatable and does not contain anti-nutrients. Corn also contains xanthophylls which [4]. The sustainable corn self-sufficiency program launched by the government is an ideal condition because Indonesia has natural resources and a supportive agroecological environment [5]. However, efforts to increase production are inseparable from biotic and abiotic environmental constraints that reduce productivity, including climate disturbances, use of poor quality seeds and attacks by plant pest organisms.

In Indonesia, it is reported that several main diseases that infect corn plants include downy mildew, maydis leaf blight, and leaf rust. Evaluation of the resistance of new high-yielding varieties to these three diseases is a major prerequisite for the release of new varieties [6]. However, at this time, pathogenic infections are starting to be found that cause tissue rot in the stalk with soft rot and slimy characteristics, plants wilt and heavy attacks cause plants to die. The literature study conducted showed that the symptoms of the disease were stalk rot caused by infection with the *Dickeya zea* bacterium, previously known as *Erwinia chrysanthemi* pv. *zea* which is one of the most important diseases of corn in the world.

Based on those, the ethological study about this disease in Indonesia need to be concern. The information about the symptom, pathogen characterization, the distribution and the impact to the maize production in Indonesia must be done. The characterization of bacteria that cause stalk rot disease in corn from pathological, biochemical and molecular characteristics to be used as a basis for handling corn stalk rot disease. Pathological characteristics were carried out to see the virulence of bacterial isolates found from corn cultivation in infecting and causing disease as well as the resistance of several existing varieties of corn to stalk rot disease. Biochemical characteristics were carried out to see the physiological properties of the bacterial isolates that cause stalk rot disease to determine the characteristics and specifics by looking at the enzyme activity. Molecular characteristics to obtain more accurate information on the bacterial species found.

2. Symptom of Disease

Symptoms of corn stalk rot disease caused by bacterial infection are generally found to be maceration of the stalk and a change in the color of the infected tissue to brown and softened rot, emitting an unpleasant odor and eventually the plant collapses [7-9]. The presence of an unpleasant odor is one of the things that distinguishes the symptoms of stalk rot disease caused by bacteria and fungi [10].

Corn stalk rot disease is economically detrimental because of the interruption of the flow of nutrients to plant tissues so that the filling of the cobs is not perfect and even severe infections can kill plants before physiological maturity [10]. Furthermore, [9] and [11] found that severe infection conditions caused infected plants to collapse, resulting in a significant reduction in yield (Figure 1). Severe infections are usually found when climatic conditions with high temperature and humidity, such as in tropical and sub-tropical regions, occur sporadically [12]. Symptoms of the disease found in one of the corn development areas in South Sulawesi are the same as the description above, if the infected corn stalk is split, the color changes to brown and emits an unpleasant odor, the plant dies as a whole (Figure 2).



Figure 1. Symptoms of bacterial stalk rot disease on corn found in Korea (Myung et al., 2010)



Figure 2. (a) Symptoms of plants infected with bacterial stalk rot disease found in Kab. Gowa, South Sulawesi in 2021; (b) Maceration of infected stalks from below the soil surface; (c) discoloration of infected stalk tissue; (d) healthy stalk tissue (Private Collection).

3. Characteristics of the Pathogen

Identification of bacteria in general can be done in several ways, including by observing morphology, biochemical properties, or by using molecular equipment. Observation of bacterial morphology can be done macroscopically and microscopically by observing the shape of the colony, such as point-shaped, round, irregular like roots and filamentous or threaded and coiled. Colony edges can be whole, wavy, split, serrated, threaded and curly. Colony color consisted of whitish, yellowish, reddish, brown, orange, pink, green and purple. Colony elevations include flat, horizontal, curved and convex. The colony structure is smooth, shiny, rough, wrinkled or curly [13].

Dickeya zeae which is one of the bacteria that causes corn stalk rot disease that has been widely reported so far. Specific identification of the presence of this bacterium is its ability to produce indigoidine, a blue pigment that is insoluble in water. *Dickeya zeae* is the only species in the bacterial genus *Erwinia* that is able to give a blue color to bacterial colonies as a chemotaxonomic property for rapid identification [14].

Morphological identification carried out by [11] against *D. zeae* found the characteristics of rod-shaped and gram-negative bacteria. Size varies from 0.8-3.20 0.5-0.8 μ m (mean 1.8 x 0.6 μ m) and contains 3-14 peritrichous flagella. These bacteria were producing white, slimy and shiny colonies on King's B media, while on Nutrient Agar media the bacterial colonies were gray and slightly prominent [9] (Fig. 1A and 1B).

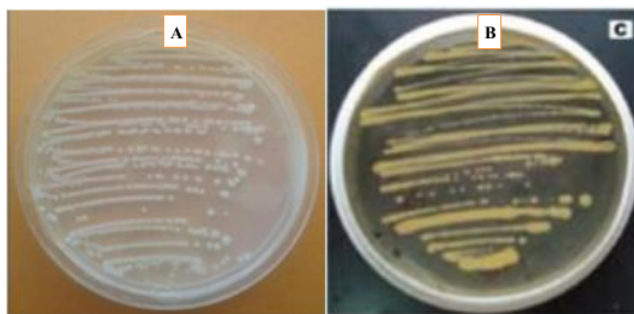


Figure 3. (A) Single colony culture of *D. zea* isolated from corn stalks and purified on King B media [11] and *D. zea* culture on Nutrient Agar media [15].

The morphological characteristics of bacteria are considered less effective because of their large potential for contamination [16]. Another drawback of this method lies in the nature of the bacteria obtained. This is because many bacteria have the same colony shape and color. Therefore, identification of bacteria needs to be done in several ways including biochemical, pathological and molecular characteristics. In particular, bacteria that cause stalk rot in corn have been identified by several researchers, both from biochemical characteristics and molecular detection. However, information regarding this bacterium in corn plants has not been widely reported in Indonesia.

3.1. Characteristics of Pathology

Bacterial pathogenicity is the ability of a pathogenic bacterium to cause disease. [17] stated that the pathogenicity of each pathogenic agent is also closely related to its ability to produce enzymes, toxins and the ability to overcome the host's immune system. In addition, bacterial pathogenicity is a multifactorial process, successful infection requires temporal coordination of survival and expression of virulence genes [18].

Dickeya zea is also known as Erwinia soft rot bacteria which belongs to the Enterobacteriaceae family. The bacteria are gram negative rod-shaped with peritrichous flagella [19]. *D. zea* produces several virulence factors including phytotoxic zeamines and extracellular enzymes including pectinase, protease, feruloyl esterase and cellulase enzymes which collectively contribute to bacterial infection [20-22]. These bacteria secrete enzymes that destroy plant cell walls, causing cell lysis and the release of cellular fluid in the form of characteristic rot symptoms [23].

Characterization of the bacterial pathology of corn stalk rot disease is usually carried out by testing the decay activity of potato tubers, hypersensitivity reactions on tobacco leaves, pathogenicity tests on corn plants and appropriate inoculation methods. Bacterial isolates that have high virulence can be used to screen corn resistance to stalk rot disease. The high virulence of the bacteria is indicated by its ability to cause soft rot symptoms that are getting faster in all infected plant tissues [24]. Soft rot bacteria from the *Dickeya* sp. will enter the potato tuber through lenticels, stolon and or wounds and the infection can spread to all parts of the plant [19].

Testing the pathogenicity of plant pathogens must be supported by appropriate inoculation techniques. [25] tested 4 methods of inoculation of *D. zea* on corn in India and the results showed that the method of stalk injection and immersion of plant roots in bacterial suspension caused the greatest incidence of disease. However, the root immersion method with bacterial suspension was considered less effective in testing plant resistance to stalk rot disease in plantations and the chance of plant damage due to carelessness during root removal was quite high. [26] reported the results of testing 6 methods of inoculation of *Dickeya dadantii* bacteria in causing sorghum stalk rot disease showing that the use of the tootpick inoculation method resulted in a fairly high incidence of disease.

3.2. Biochemical Characteristics ²

Biochemical tests help identify different bacterial species based on different biochemical activities. Differences in carbohydrates, proteins, fat metabolism, production of certain enzymes, ability to utilize certain compounds etc., help to identify microorganisms. Several researchers have identified biochemically *D. zeae* isolated from corn plants and biochemical characteristics such as facultative and non-fluorescent anaerobic, pectinolytic in potato tubers, causing hypersensitivity reactions in tobacco leaves, producing catalase and lecithinase, not producing oxidase or arginine dehydrolase, capable of reducing nitrate and can grow at a temperature of 37°C. Among the 23 strains studied, 11 grew well under 5% NaCl, while the growth of the other 12 strains was inhibited, indicating that some bacterial strains were more tolerant of salt [27]. The strain formed intensive "fried egg" red colonies with a diameter of 1.5 mm on potato dextrose agar (PDA) medium (Table 1). These growth characteristics are described as typical for bacteria belonging to the genus *Dickeya*.

Table 1. Physiological and Biochemical Characteristics of *Dickeya zeae* isolated from maize and the reference strain.

Characteristic	Strains of <i>Dickeya Zeae</i> (n=23)	Reference strains		
		<i>Dickeya</i> spp. (KBI 05)	<i>Pcc</i> (KFB 85)	<i>Pba</i> (KFB 07)
Gram reaction	-	-	-	-
HR on tobacco leaves	+	+	+	+
Oxidase activity	-	-	-	-
Catalase activity	+	+	+	+
Lecithinase activity	+	+	+	+
Fluorescence on KB	-	-	-	-
Glucose metabolism	OF	OF	OF	OF
Potato soft rot	+	+	+	+
Growth at 37 °C	+	+	+	-
Growth in 5% NaCl	v	+	+	+
Growth on Logan's medium	+ ^a	+ ^a	+ ^b	+ ^c
"Fried egg like colonies on PDA medium	+	+	+	+
Nitrate reduction	+	+	+	+
Pathogenicity assay	+	+	+	+

Legend : + indicates positive reaction; - indicates negative reaction; v, indicates variable reaction; OF, oxidative-fermentative metabolism of glucose; +^a intensive red colonies 2 mm in diameter, +^b, smaller (1,5 mm) colonies with pink center, +^c, small (<1.5 mm) white-grey colonies

Source : [27]

Other researchers reported the same characteristics of the bacteria causing corn stalk rot found in Korea. These bacteria are gram-negative, oxidase negative, catalase positive, fermentative, rod-shaped, motile, and facultative anaerobes. The results of biochemical tests performed using the Biologic Microbial Identification System, version 4.2 (Biolog Inc., Hayward, CA) showed that all isolates had a similarity index of 0.65 to 0.73 with *D. zeae* [9].

Dickeya zeae in addition to infect corn was also found to infect horticultural crops such as pineapple. The results of the identification of bacteria that cause rot in pineapples identified as *D. zeae* were reported to be gram negative, soft rot, facultative anaerobes and virulent [28].

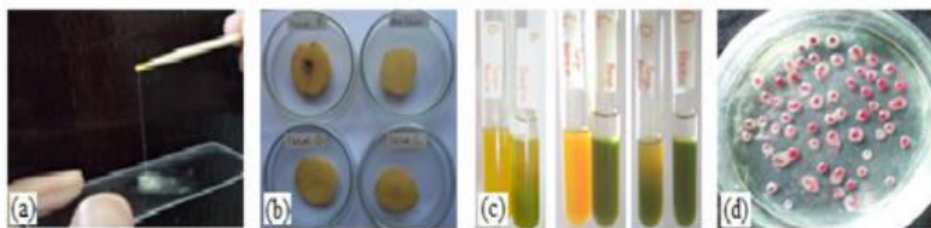


Figure 4. (a) Gram test results using 3% KOH, (b) and (c) Potato tuber rot test results (left) and O/F test on bacterial isolates B, C and D (right) Test results using TZC media on isolate B, (d) The results of the test using TZC media on isolate B [28].

2.3. Molecular Characteristics

Molecular characterization is an important tool for the identification of plant pathogens with the help of locus/gene-specific primers [29-30]. Strategies that are widely used for rapid detection of pathogens include screening target DNA sequences or using probes to develop DNA markers of infecting pathogens [31], as well as molecular detection with simple Polymerase Chain Reaction (PCR) and its development.

Molecular test with PCR is a technique of DNA synthesis and amplification in vitro. This technique was discovered by Kary B. Mullis and F. Faloon in 1985. PCR is based on enzymatic amplification of DNA fragments using two complementary oligonucleotide primers with the 5' ends of both strands of the target sequence. These oligonucleotides are used as primers (PCR primers) to allow DNA templates to be copied by DNA polymerase [31].

The genus *Dickeya* as a bacterium that causes soft rot disease in many plants consists of six species, namely *D. dianthicola*, *D. dadantii*, *D. zea*, *D. chrysanthemi*, *D. dieffenbiana* and *D. paradisiaca* [23]. The results of biochemical characteristics conducted by [32] stated that *D. dadantii* and *D. zea* are in the same phenon so that molecular identification is needed for more accurate determination of pathogenic species. Their research to identify the bacteria that causes soft rot in pineapple plants used three different genes, namely 16S rDNA, *recA*, and *dnaX*. The 16S rDNA approach is considered as one of the most widely used standard techniques for inferring phylogenetic relationships among bacteria but sometimes it is not sufficient to distinguish closely related species. While *recA* and *dnaX* genes have been shown to be strong markers for inferring bacterial phylogeny and have been used successfully to differentiate *Dickeya* species; [23]; [33-34]; [13].

The use of specific primers for the detection of *D. zea* was first carried out by [35] used a specific primer set (ADE-1, ADE-2) to detect 78 strains of *D. zea* and all markers showed a specific band of 420 bps (Figure 3). Until now, this primer has been widely used for the detection of *D. zea* in several commodities such as pineapple, aloe vera and orchids.

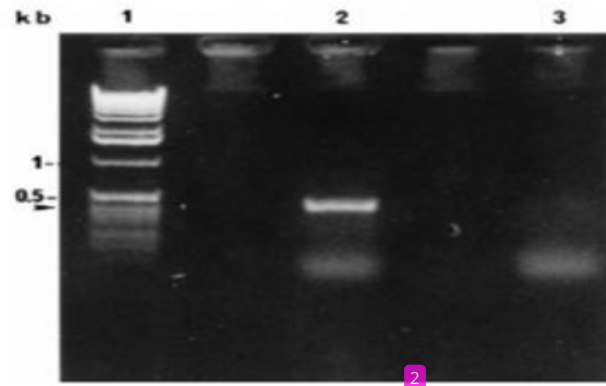


Figure 5. Results of PCR amplification using specific primers ADE1 and ADE2. The PCR products (after 25 cycles) were separated by electrophoresis on 1% agarose gel. Lane 1, 1-kb DNA ladder; lane 2, *E. chrysanthemi* 3937. The arrow direction indicates the position of the 420-bp amplified fragment [36].

Molecular identification of *D. zea* carried out by [9] isolated from corn plants in Korea showed the results described in the following phylogenetic tree (Figure 4). There are two isolates isolated in Korea, namely *D. zea* BC2879 and *D. zea* BC2880 which have a close relationship with the isolates *D. zea* IPO649, *D. zea* NCPPB1863 and *D. zea* NCPPB2538. Meanwhile, the phenotypic test and amplification of the specific 420-bp fragment in the PCR test conducted by [27] showed that 7 isolates of corn stalk rot were included in the *Dickeya* genus based on phylogenetic analysis based on gene sequence results using recA. Using ERIC-PCR analysis seven different genetic profiles were obtained, indicating the presence of genetic diversity in the population of this pathogen in Serbia.

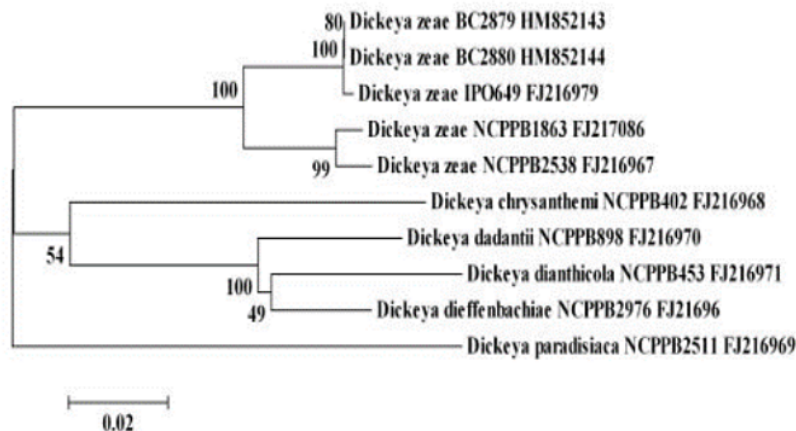


Figure 6. Phylogenetic tree of PCR test results of 2 isolates of corn stalk rot pathogen found in Korea [9].

4. Disease Distribution

This maize stalk rot disease firstly reported by Prasad in 1930. The identification showed that this disease caused by *Erwinia dissolvens*. However, the characteristic tends to *Erwinia chrysanthemi*. The outbreak of this disease happened in Himachal Pradesh, 1969. The pathogen spread from one to other plant through the rain and the water flow.

This bacterium has long been found to infect corn plants in the Philippines with high attack intensity [10]. *D. zea* was also reported to attack hybrid and composite corn in four corn growing areas in India with an average disease incidence of 96.65% [25]. Apart from these two countries, *D. zea* was found to infect corn crops in Nepal, Serbia, China and Mexico in the last 10 years [27]; [36]; [12]; [8]. Initial infection in Shanghai China was found to attack sweet corn [8]. Until now, information regarding the detailed identification of bacteria that cause stalk rot disease in Indonesia is still very limited. Stalk rot disease that has been reported in Indonesia is caused by the fungus *Fusarium* spp. [37]. In the Minister of Agriculture No. 25 of 2020, this bacterium is classified in OPTK A2, which means that it is already present in Indonesia, but is limited to certain areas. The presence of *D. zea* has been found to attack aloe, pineapple and corn plants in Indonesia [28]; [38-40]. This bacterial infection was found to attack pineapple plantations in the Lampung region and the host range test conducted by [32] showed that the isolates of *D. zea* bacteria isolated from pineapples and inoculated on corn plants showed symptoms of stalk rot. The presence of *D. zea* infecting corn plants in the West Sulawesi region was first reported by the Mamuju Class II Quarantine Station in 2019. The results of molecular identification showed that the bacteria found was *D. zea* [40]. Meanwhile, other maize production areas have not do the identification and the survey about the disease distribution.

5. The Impact to the Maize Production in Indonesia

This maize stalk rot disease caused yield loss production directly by affect the plant physiological. This disease attack will caused the plant collapse and affect to the economic value [41]. High humidity factors and low oxygen levels cause high disease incidence and spread, pathogenic infections increase because they are facultative anaerobes (Perambelon, 2002 in [19]). This pathogen can be transmitted through the soil [42] but the inoculum can persist in infected plant residues in the soil for 270 days [43]. These bacteria can move in the soil up to a distance of 10 m through free water so that they can infect surrounding plants. In addition, the spread of bacteria can also be assisted by vectors in the form of insects from infected plants to healthy plants and can appear in aerosols formed by impaction of rain on symptomatic plants. These bacteria can also survive in surface water and can be spread through irrigation water (Lauria et al. 2008 in [19]). Another factor that triggers the development of this pathogen in agricultural land is numerous host plant.

6. Conclusion

The maize stalk rot caused by bacteria need to be concern because of the economic loss impact. The bacteria that caused stalk rot disease in corn reported from several countries are generally identified as *Dickeya zea*, including those found in one of the 40m development areas in Indonesia. The bacteria are gram negative, facultative anaerobes, produce catalase and lecithinase, do not produce oxidase or arginine dehydrolase, are capable of nitrate reduction and can grow at 37°C, producing several plant cell-degrading enzymes, pectinolytic properties on potato tubers and causing hypersensitivity reactions in tobacco leaves. The spread of bacteria through the soil, the ability to survive on infected plant residues for a long time and a wide host range can be a threat factor in the spread of this pathogen in Indonesia.

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