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Damaging Power of Bacillus Sp. From The Soil of Liwa Botanical Garden As A Control Agent Against Dickeya Spp. A Cause of Disease In Plants

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Abstract: *Bacillus is one of the bacteria that has the potential as a biological agent to inhibit plant-disturbing organisms, such as Dickeya* spp. *The purpose of this study was to determine the inhibition of Bacillus sp. isolates isolated from the soil of the Liwa Botanical Garden related to its ability as a biological control agent against Dickeya spp. The design used in this study was a completely randomized design (CRD) with 3 replicates. This research includes several stages, namely propagation of Bacillus sp. bacteria, Dickeya spp. pathogenic bacteria, enzymatic tests of Bacillus sp. bacteria and antagonist tests. In this study, all Bacillus sp. isolates showed positive results in the Protease, Cellulase and Lipase Enzyme Test and all Bacillus sp. isolates showed negative results in the chitinase enzyme test. In the antagonist test, all Bacillus sp. isolates code TBA7, TSR6 and TSR 5 showed positive test results characterized by the presence of a clear zone in the agar diffusion disk test.*

Keywords: *Antagonist, Bacillus sp., Dickeya spp*

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I. INTRODUCTION

Liwa Botanical Garden is an ex-situ plant conservation area, located in Kubu Perahu pekon, Balik Bukit sub-district, Liwa, West Lampung Regency, Lampung Province. Liwa Botanical Garden has a large collection of plants, both in seedlings, nurseries and those that have been planted. This plant collection is divided into six garden groups, namely the fruit garden, ornamental garden, araceae garden, aren palm garden, fragrant thematic garden / ornamental mother garden, and rainbow garden.

In crop cultivation, efforts cannot be separated from various obstacles. Plant Disturbing Organisms are one of the main obstacles in this crop cultivation. Plant Disturbing Organisms (pests) can be in the form of pests or pathogenic microbes that cause disease in plants. One of the plant pest organisms that must be aware of its spread in plants, *Erwinia chrysanthemi*, is currently reclassified as *Dickeya* (Haerani et al., 2015). *Dickeya* spp. was first reported by Muhammad Machmud in 1985 as the cause of potato blight in Java (Semangun 1989). Supriadi et al. (2002) also reported that *Dickeya* spp. was the cause of decay on the leaves and stem base of aloe vera plants in Semplak, Bogor, West Java. Apart from aloe vera plants, *Dickeya* spp. also causes soft rot disease in Phalaenopsis orchids in DKI Jakarta and West Java (Muharam et al. 2012). Oviana et al. (2015) have also reported that *Dickeya* spp. can cause soft rot on pineapple fruit in the plantation of PT Nusantara Tropical Farm.

Efforts to control Plant Disturbing Organisms (OPT) that are environmentally sound in reducing the negative effects of chemical pesticide compounds need to be developed, one alternative method that can be applied is to use biological agents that are safer and environmentally friendly because the control organisms used can come from nature itself and their activities can be modified according to the new environment according to the desired plant. The use of biopesticides made from active bacteria to control plant pathogens has also begun to be used. One of the bacteria that has potential as a biopesticide and is safe in plant management is the genus Agrobacterium and *Bacillus* (Beric et al., 2012).

Based on the results of research by Nawangsih et al. (2009), biopesticides made from *Bacillus subtilis* isolate number B12 and isolate Pf10 formulated in a solution of vermicompost and molasses can control pathogens that cause disease in Phalaenopsis orchids. This allows *Bacillus* sp. bacteria to have potential as biological control

agents. *Bacillus subtilis* is known to be able to control several pathogens, both fungi and bacteria, by inhibiting their growth (Chen et al., 2012).

The control mechanism by *Bacillus subtilis* as a direct activity is in the form of antibiosis, parasitism and induction of plant resistance to pathogens and direct competition (Janisiewicz et al., 2000). The mechanism of antibiosis is seen with the formation of an inhibition zone, which indicates the presence of a compound produced as a secondary metabolite and functions as an antibiotic activity. These compounds are enzymes, toxins, and antibiotics (Prihatiningsih & Djatmiko, 2014). Based on research by Morikawa (2006), *Bacillus subtilis* secretes amylase, protease, pullulanase, chitinase, xylanase, and lipase enzymes. *Bacillus subtilis* can also produce antibiotics such as Bacillomycin D and iturin produced by *Bacillus subtilis* AU195 and QST713 (Prihatiningsih, et al., 2015).

The *Bacillus* genus shows an antibiotic mechanism in the suppression of pathogenic bacteria, by producing inhibitory compounds, also called antimicrobial compounds, including antibiotics, phenol compounds and enzymes, alkaloids and siderophores (Haggag & Mohamed, 2007). Siderophores are one of the compounds produced by antagonistic bacteria that mediate competition in chelating iron, so that it is antagonistic because the pathogen becomes deficient in iron as a nutrient. Siderophores produced by *Bacillus* have biocontrol implications for pathogenic fungi and bacteria such as *Penicillium chrysogenum, Fusarium oxysporum, Rhizoctonia solani* and *Clavibacter michiganensis* (Yu et al. 2011). Siderophores also benefit plants because they form siderophore-Fe bonds that become plant-available forms of Fe. Siderophores are low molecular weight compounds capable of chelating iron (Fe3+), and are responsible for the solubilization and transport of this element into bacterial cells (Sharma & Johri, 2003). Under conditions of iron limitation, siderophore-producing microorganisms can bind and transport siderophore-iron complexes by expressing specific proteins (Nudel et al., 2001). Siderophores produced by bacteria are one of the antibiotic mechanisms against pathogens, with orange zones formed on SD-CASA medium (Prihatiningsih et al. 2017). In addition, according to Choudhary & Johri (2008), biological agents such as *Bacillus* spp. can act as biological fertilizers and biological control agents through antibiosis mechanisms, secretion of lyse enzymes, and induction of systemic resistance.

II. EXPERIMENTAL PROCEDURE

In this reserch, the Antagonist Test of *Bacillus* sp. bacteria produced from soil isolation of Liwa Botanical Garden and *Dickeya* spp. bacteria was carried out by using the Agar Diffusion disk method based on research (Radjasa et all, 2007). The design used in this research is a completely randomized design (CRD) with negative control treatment using distilled water. The study began with the subculture stage (Rejuvenation) of bacterial isolates to be used, namely *Bacillus* sp. with isolate codes (TSR5, TSR6, & TBA7) and *Dickeya* spp. Then continued with biochemical tests (chitinase, protease, cellulase and lipase enzyme tests) against *Bacillus* sp. bacteria, after which it was continued with the antagonistic test between *Bacillus* sp. vs *Dickeya* spp. bacteria, which were then incubated at room temperature for 24-48 hours and then the clear zone formed**.** was observed.

1.1 **Subculture of *Bacillus* sp. And *Dickeya* spp. isolates**

Subculture (Rejuvenation) Isolates of *Bacillus* sp. obtained from the isolation of Liwa Botanical Garden soil and *Dickeya* spp. obtained from the isolation of the Plant Protection Laboratory, Faculty of Agriculture, University of Lampung, were carried out using Nutrient Agar (NA) media, tilted in a test tube. Then the sterile media was poured into a test tube and tilted, and then allowed to solidify. After that, 1 isolate was taken using a round ose and then scratched (streak method) on a test tube containing NA media that had been solid and then incubated at a temperature of ± 37ºC (in an incubator) for 24 hours.

1.2 **Enzymatic Test of *Bacillus* sp.**

Chitinase Test: The chitinase test was carried out by growing *Bacillus* sp. bacteria on 0.3% chitin agar medium (KH2PO4 0.02%, $MgSO_4$, $7H_2O$ 0.1%, colloidal chitin 0.3%, yeast extract 0.1%, and 2% agar). Then, *Bacillus* isolates were inoculated on 0.3% chitin agar medium and then incubated for 4 days at room temperature. Positive results are characterized by a clear zone around the colony (Nurdin et al., 2015).

Protease Test : In the protease test, bacterial isolates were rejuvenated on Nutrient Agar (NA) media for 24 hours at 37°C. Then for the qualitative test of protease enzyme activity was carried out by inoculating *Bacillus* sp. isolates using the dot method on skim milk agar media. Positive results are indicated by the presence of a clear zone around the bacterial colony.

Lipase Test: In the lipolytic activity test, *Bacillus* isolates were identified by qualitative tests using lipase selective media with the content of ingredients per liter, namely (5% sterile olive oil, 2.5% Tween-80, 5g NaCl, 0.1g CaCl2.2H2O, 0.01% methyl red, 10g peptone, and Nutrient Agar). The 24-hour-old *Bacillus* isolates were

inoculated using the point method on selective media. Then isolated for 24-48 hours at room temperature with the cup upside down. Positive results are characterized by the presence of a clear zone around the bacterial colony (Bestari and Suharjono, 2015).

Cellulase Test: The cellulase test was carried out by inoculating 24-hour-old *Bacillus* isolates using the dot method on 1% NA + CMC media. Furthermore, it was incubated for 24 hours, and then the media was flushed using 2 M NaCl. For positive results indicated by the clear zone (Sumardi et al., 2018)

1.3 **Antagonistic test of *Bacillus* sp. against pathogenic bacteria *Dickeya* spp.**

Antagonism test of *Bacillus* sp. isolates against plant pathogenic bacteria *Dickeya* spp. was conducted using agar diffusion method on media (NA) based on research (Radjasa et all, 2007). Pure isolates of *Bacillus* sp., *Dickeya* spp. were each diluted and compared with the McFarland 0.5 standard solution based on Quelab (2005), (McFarland 0.5 is equivalent to 1 x 107 - 1 x 108 cells/ml). After that, 0.1 mL of suspension from the Nutrient Agar dilution was taken and leveled on a Petri dish containing solid Nutrient Agar (NA) media. Furthermore, a sterilized paper disc measuring 0.5 cm was dipped into the suspension of *Bacillus* sp. bacterial isolates, which had also been diluted and compared with McFarland 0.5 standard solution, sterile distilled water as a negative control and Chloramphenicol as a positive control. Furthermore, the Papprer disc that has been dipped in the suspension is placed on solid NA media that has been swabbed with a suspension of pathogenic bacterial isolates (*Dickeya* sp.) in the centre of the cup, then incubated for 24-72 hours in an incubator at 37ºC. After that, it was observed whether or not there was an inhibition zone/clear zone that occurred. Tests were carried out on each bacterial treatment in duplicate, with a repetition of 3 times, on the media.

III. RESULTS AND DISCUSSIONS

Based on the results of the enzymatic test of *Bacillus* sp. isolated from the soil of the Liwa Botanical Garden, obtained results indicate the presence of enzyme activity as indicated by the clear zone formed in the 10 *Bacillus* sp. isolates. The most optimal enzymatic activity is in the protease enzyme test, which shows that all isolates have clear zones (or have protease enzyme activity) while for less optimal enzyme activity there is a chitinase enzyme test because there is only one isolate that forms a clear zone. The results of the enzymatic test can be seen in Table 1.

Table 1. Enzymatic Test Results of *Bacillus* sp. Isolates in Liwa Botanical Garden Soil

Isolate Code	Chitinase Enzyme	Protease Enzyme	Lipase Enzyme	Cellulase Enzyme
TBA 7	-	+	+	+
TSR 5	-	+	+	+
TSR 6	-	+	-	+

Isolate code: TBA=Common ground Araceae; TSR=leaf litter soil

In the protease enzyme test, *Bacillus* sp. showed that all *Bacillus* isolates showed positive results or could produce protease enzymes so that they could inhibit the growth of pathogenic bacteria *Dickeya* spp. *Bacillus* can produce protease enzymes that can inhibit the growth of pathogenic bacteria through several mechanisms, such as protein digestion, Pathogenic bacteria often require protein for their growth and reproduction. Protease enzymes can hydrolyze proteins that are essential for these bacteria, making it difficult for the bacteria to obtain the resources needed to survive and reproduce. Protease enzymes can also damage important structural proteins in the cell wall of pathogenic bacteria or in their internal structure. This can lead to structural weakness or overall cell damage, inhibiting the bacteria's ability to maintain cell integrity and vital functions. Some pathogenic bacteria produce toxins that are important for their pathogenicity. Protease enzymes can decompose these toxins, reducing the ability of bacteria to cause damage to their hosts. Protease enzymes may also play a role in inhibiting the adhesion and invasion of pathogenic bacteria into host cells. By destroying proteins involved in adhesion or invasion, protease enzymes can block the ability of bacteria to enter and infect host cells (Patel et al., 2014).

In the lipase enzyme test *Bacillus* sp. also obtained all isolates showed positive results in the cellulase enzyme test. Cellulase enzyme also has an important role in inhibiting the growth of pathogenic bacteria, mainly because of its ability to break down cellulose, which is the main component of plant cell walls and some bacteria. The cellulase enzyme produced by *Bacillus* sp. can inhibit the growth of pathogenic bacteria through several mechanisms because cellulase enzymes can hydrolyze cellulose in the cell walls of pathogenic bacteria that contain cellulose components. This causes structural damage to the cell wall, weakening cell integrity and disrupting the ability of bacteria to survive and reproduce (Megala R. et al. 2016).

In addition, cellulase can also break down the main resources needed for the growth of pathogenic bacteria, namely the cell wall and extracellular matrix, cellulase enzymes can effectively inhibit the growth and reproduction of these bacteria. Cellulase enzymes can also interfere with the formation or damage biofilms formed by pathogenic bacteria. Biofilms are protective layers composed of bacteria bound to surfaces, and cellulose can

be one of the main components in the biofilm matrix (Usha et al. 2014). By breaking down cellulose, cellulase enzymes can inhibit the formation or disrupt the stability of biofilms, making bacteria more vulnerable to attacks from the immune system or antimicrobial agents. Cellulose may also play a role in the attachment of pathogenic bacteria to host or other surfaces. By damaging the cellulose layer that allows bacteria to bind, cellulase enzymes can reduce the ability of bacteria to adhere and initiate infection (Vijayalakshmi G. et al. 2016).

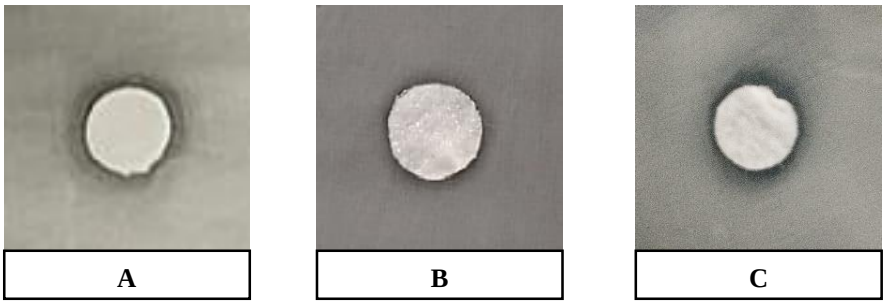
In the results of the chitinase enzyme test, *Bacillus* sp. isolates did not show positive results for this chitinase enzyme test. All *Bacillus* isolate samples cannot produce the chitinase enzyme.

3.1. Antagonistic Test of *Bacillus* sp. Isolates Against *Dickeya* spp. Bacteria

The results of the antagonistic test of *Bacillus* sp. isolates against *Dickeya* spp. pathogenic bacteria are presented in the form of macroscopic observation data related to the clear zone produced in each isolate. The results of the *Bacillus* sp. antagonist test against *Dickeya* spp. bacteria after incubation for 24 hours have different inhibitory abilities in each *Bacillus* sp. The observations of the antagonist test of 3 *Bacillus* sp. isolates with isolate codes (TSR5, TSR6, and TBA7) against *Dickeya* spp. pathogenic bacteria obtained the following results:

The results of the antagonistic test of *Bacillus* sp. bacteria against *Dickeya* spp. bacteria all showed positive results with indicators of clear zones formed, but with different inhibitory abilities characterized by different diameters in each isolate. In the *Bacillus* sp. isolate test, isolate code TBA 7, and TSR 6 produce a clear zone that is quite extensive (has a high potential ability to inhibit the growth of *Dickeya* spp. bacteria), while *Bacillus* sp. with isolate code TSR 5 also produces a clear zone with a medium diameter or it can be interpreted that the isolate also has enough potential in inhibiting the growth of *Dickeya* spp. but not very effective.

Figure 1. Antagonistic test results of *Bacillus* sp. TBA 7 (A), TSR 6 (B), TSR 5 (C) isolated from the soil of Liwa Botanical Garden against *Dickeya* spp bacteria.



Based on the results of antagonistic tests that have been carried out *Bacillus* sp. has some ability to inhibit the growth of pathogenic bacteria such as *Dickeya* spp. *Bacillus* has several mechanisms that can be used by *Bacillus* in inhibiting the growth of *Dickeya* spp. Waleron et al. 2019 reported that *Bacillus* produces various types of antibiotics that can inhibit the growth of pathogenic bacteria such as *Dickeya* spp. and Pectobacterium spp. These antibiotics can damage cell structure, inhibit protein synthesis, or disrupt other vital processes in *Dickeya* spp. Bacitracin works by binding to and inhibiting the activity of the enzyme-translocase responsible for the formation of peptidoglycan, a major component of the bacterial cell wall. Peptidoglycan is an important layer that provides strength and integrity to the bacterial cell wall. By inhibiting the formation of peptidoglycan, bacitracin causes the bacterial cell wall to become weak and unstable. In addition to inhibiting the formation of peptidoglycan, bacitracin can also increase the permeability of bacterial cell membranes. This can cause leakage of ions and essential substances from inside the bacterial cell out, as well as the entry of toxic compounds from outside the bacterial cell into it. As a result, bacterial cell function is disrupted and growth is inhibited. Bacitracin also has a bactericidal effect that can kill pathogenic bacteria directly by disrupting bacterial cell function and metabolism. This is different from bacteriostatic antibiotics, which only stop the growth of bacteria without killing them. The mechanism of inhibiting the growth of pathogenic bacteria by bacitracin makes it an effective antibiotic agent in controlling bacterial infections in plants. Yu-liang, (2014)

Besides producing antibiotics, Osuntokun et al. (2020) also reported that *Bacillus* sp. compete with pathogenic bacteria for resources and living space. *Bacillus* sp. can occupy and utilize living space in the soil environment or plant rhizosphere, thus reducing the space and resources available to pathogens. *Bacillus* sp. can also form biofilms or produce exopolysaccharides that help them to compete

with and inhibit the growth of pathogenic bacteria, *Bacillus* sp. also compete with in nutrient resources required for growth and metabolism because *Bacillus* sp. can consume nutrients more efficiently, thereby reducing the availability of nutrients for pathogenic bacteria and inhibiting their growth, and *Bacillus* can multiply rapidly, so it can inhibit growth, survival and cause a relative decrease in *Dickeya* spp. population.

IV. CONCLUSION

All *Bacillus* sp. isolates isolated from the soil of Liwa Botanical Garden showed positive results in the antagonist test against *Dickeya* spp. bacteria (potential in inhibiting the growth of *Dickeya* spp. bacteria) with different abilities in each isolate. *Bacillus* sp. isolates with code TBA 7, and TSR 6, have the highest inhibitory power (producing the widest clear zone) in inhibiting the growth of *Dickeya* spp..

Conflict of interest

There is no conflict to disclose.

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