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ANTIBACTERIAL ACTIVITY OF *Bacillus sp* ISOLATED FROM LIWA BOTANICAL GARDEN SOIL AGAINST *Dickeya sp.*

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ABSTRACT

The soil *Bacillus* group has been reported to produce antibacterial compounds. One of the plant pathogenic bacteria is *Dickeya sp.* which causes A strain of *Bacillus* bacteria from the soil of the Liwa Botanical Garden (KRL) has been successfully found and showed antibacterial activity against *Dickeya sp.* The *Bacillus* strain has been characterized as *Bacillus vallismortis* RKB1 strain through 16S rRNA phylogenetic analysis. This study aims to determine the activity of antibacterial compounds of *Bacillus* from KRL soil against *Dickeya sp.* Crude extract produced from liquid culture of *Bacillus vallismortis* RKB1 on antibacterial production medium showed antibacterial activity against *Dickeya sp.* Separation of compounds from crude extract by open column chromatography produced 8 fractions and there were 3 active fractions that showed inhibition against *Dickeya sp.* in the antibacterial activity test through agar disc diffusion. Fraction 54 showed the greatest inhibition and was classified as strong with an inhibition zone of 13.5 mm at a stock concentration of 5000 µg/ml. The fraction has a Minimum Inhibitory Level (KHM) at a concentration of 500 µg/ml based on turbidity value and resazurin staining through liquid microdilution. The identification results of Thin Layer Chromatography (KLT) on the active fraction with eluent n-hexanaana: ethyl acetate (7:3) showed a polar complex .This indicates that the compounds in the active fraction contain peptide compounds.

Introduction

Liwa Botanical Garden (KRL) has a diverse collection of ornamental plants and has found cases of soft rot disease caused by pathogenic bacterial infections [1]. Plant pathogenic bacteria, one of which is the genus *Dickeya*, is known to cause soft rot disease in agricultural crops and ornamental plants almost all over the world [2][3]. It has been reported that *Dickeya* fangzhongdai infection caused leaf soft rot disease in some epiphytic orchids in Vietnam [4]. *Dickeya* infects plants through open parts of the epidermal tissue, then secretes the enzyme pectinase which can degrade pectin in plant cell walls [5][2]. This results in tissue softening and organ decay of the host plant [6].

Studies related to the control of soft rot disease caused by *Dickeya* genus bacterial infection have been widely reported. However, many plant disease treatments are still carried out using chemical methods without considering the negative impacts on the environment and living things [7]. One alternative control that is environmentally friendly is biological control with soil microorganisms, such as *Bacillus* . *Bacillus amyloliquifaciens* and *Serratia plymuthica* have been utilized as biological agents in controlling the genus *Dickeya* [5][8]. *Bacillus* is a Gram-positive bacterium that has endospores [9][10] and is the dominant bacterium in soil [11]. The presence of soil *Bacillus* has also been found in KRL soil [12]. Previous research related to indigenous *Bacillus* as a biocontrol agent against *Dickeya* has been reported by [13]. The *Bacillus* genus from soil is known to have the ability to produce lipopeptide antibacterial compounds, including surfactin, iturin, and fengycin, which are widely studied regarding their antagonistic activity in inhibiting phytopathogenic bacteria [14][15][16]. The lipopeptide compound surfactin from *Bacillus* amyloliquefaciens A3 is able to inhibit the

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phytopathogenic bacteria *Dickeya* dadantii [5]. Antibacterial compounds produced by indigenous *Bacillus* in KRL in inhibiting the growth of *Dickeya sp*. are not yet known. Based on this, this study aims to determine the antibacterial activity of compounds produced by *Bacillus sp*. from KRL soil against plant pathogenic bacteria of the genus *Dickeya*.

Methods

Bacterial Isolate and Inoculum Preparation

The *Bacillus* isolate used was *Bacillus sp*. with the code TSR 6. *Bacillus sp*. TSR 6 isolate is a collection from the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, which has been successfully isolated from the litter soil of the Liwa Botanical Garden, West Lampung. Previously, the isolate has been known to have antibacterial activity with the largest inhibition zone in the antagonistic test against *Dickeya sp*. 24-hour-old *Bacillus sp*. TSR 6 was inoculated as much as 2 ose in 50 ml of NB media (pH 7). The inoculum was made in three replicates and incubated for 24 hours in a shaker incubator at 120 rpm.

Production of Antibacterial Compounds

Bacillus sp. TSR 6 inoculum of 50 ml was inoculated into 300 ml liquid antibacterial production media with the composition (g/L): glutamic acid 5 g; KH_2PO_4 0.5 g; $MgSO_4.7H_2O$ 0.2 g; MnSO4.H2O 0.01 g; FeSO4.7H2O 0.01 g; CuSO4.7H2O 0.01 g; CaCl2.2H2O 0.015 g; glucose 10 g; and pH 7 [17]. Antibacterial production was made in three replicates for a production scale of 1 liter, then incubated for 5 days in a shaker incubator at 120 rpm.

The harvesting process was done by centrifugation at 7000 rpm for 10 minutes at 4oC. The culture was divided into 25 ml into 50 ml sterile centrifuge tubes. The supernatant was taken and concentrated to 100 ml with a rotary evaporator.

Extraction of Antibacterial Compounds

The antibacterial extraction process was carried out by partitioning using a 250 mL separator funnel. Partitioning is done in stages three times with a ratio of 1: 1 (water fraction: ethyl acetate). The results of the partition will form the separation of two fractions, namely the water fraction and the ethyl acetate fraction. The yellowish ethyl acetate fraction was collected in a 100 ml Erlenmeyer, then the solvent was evaporated and the dry extract was weighed. The extract was reconstituted with ethyl acetate and stored in a 5 ml vial bottle. In the previous study, the antibacterial activity of the crude extract of *Bacillus sp*. TSR 6 against *Dickeya sp*. Furthermore, the separation of compounds by open-column chromatography was carried out.

Separation of Antibacterial Compounds by Open Column Chromatography

Separation of antibacterial compounds from crude extracts was carried out by open column chromatography using silica gel 60 stationary phase (0.2-0.5 mm) and mobile phase in the form of several types of solvents. The elution method used was the gradient elution method with several different solvent compositions. The gradient eluents used in this study were: n-hexane (100%); n-hexane:ethyl acetate (7:3); n-hexane:ethyl acetate (1:1); ethyl acetate (100%); ethyl acetate:isopropanol (1:1); isopropanol (100%); and ethanol (100%). The fractions that have been separated are each carried out KLT analysis with silica gel F254 and 100% ethyl acetate eluent, then reacted with cerium sulfate reagent to determine the presence of fractionated compounds. If it is known that there are fractions that have the same stain separation pattern, they are combined into 1 fraction.

Activity Test of Antibacterial Compound Candidates against *Dickeya sp.*

The fractions of antibacterial compound candidates that have been obtained are then screened for antibacterial activity against *Dickeya sp*. by agar disc diffusion method. A 24-hour-old suspension of *Dickeya sp*. pathogenic bacteria was inoculated several ounces into 9 ml of sterile 0.85% NaCl and homogenized. The turbidity of the suspension was adjusted to the equivalent of Mcfarland standard 0.5 with a cell density of 1x108 CFU/ml. Turbidity values were measured with a Hospitex plate reader at a wavelength of 600 nm. The suspension that was in accordance with the Mc. Farland 0.5 was taken as much as 0.1 ml and then diluted in 9.9 ml of 0.85% NaCl or MHB media to obtain a cell density of 1×106 CFU/ml. The test bacterial suspension (1×106 CFU/ml) of 0.1 ml was inoculated and swabbed on the surface of Mueller Hinton Agar media (2 g beef extract; 17.5 g acid hydrolysate of casein; 1.5 g starch; and 17 g agar (g/L)) [18].

Each stock of candidate antibacterial compounds at a concentration of 5000 µg/ml, positive control (chloramphenicol) 5000 µg/ml, and negative control (MeOH) was dripped as much as 150 µl on a paper disk with a diameter of 0.5 cm and waited until it dried. Each dried paper disk was placed on the surface of the agar and made as many as 3 replicates in another Petri dish, then incubated for 24 hours at 37ºC. Antibacterial activity is characterized by the presence of a clear zone around the disc paper. The clear zone formed was then measured in diameter with a 30 cm ruler and entered into the inhibition zone calculation formula [19].

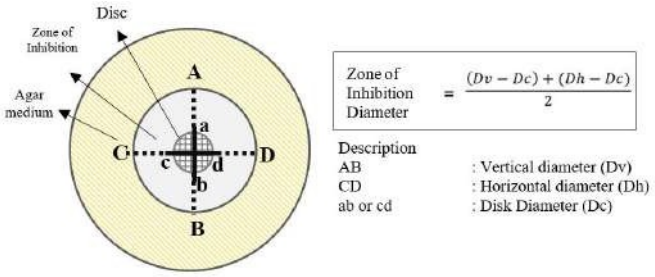


Figure 1. Inhibition zone calculation formula [19].

The zone of inhibition that has been measured is then categorized as inhibition, namely if <5 mm is classified as weak; 5-10 mm is classified as medium; 10-20 mm is classified as strong; and >20 mm is classified as very strong [20].

Preliminary Identification of Antibacterial Compounds in the Active Fraction by KLT

The fraction that was active in inhibiting *Dickeya sp*. was then subjected to KLT to determine its compound components. The eluent used was N-hexane:ISOPROPANOL (7:3) and stationary phase silica gel 60 F254. Visualization under UV λ 254 nm and reaction with cerium sulphate reagent to identify the presence of organic compounds and reacted with ninhydrin reagent to determine components containing peptide bonds. This is related to lipopeptide compounds that show positive purple results when reacting with ninhydrin [21]. Retardation factor (Rf) value of each stain is calculated to determine the polarity level of each active fraction. The Rf value can be defined as follows [22].

$$\mathrm{Rf} = \frac{\text{compound} - \text{driven distance}}{\text{Solvent} - \text{driven distance}}$$

Determination of Minimum Inhibitory Concentration (MIC) by Micro dilution

After obtaining the active fraction, then each active fraction in inhibiting *Dickeya sp*. determined the Minimum Inhibitory Concentration (MIC) through liquid micro dilution with MHB media and 96-hole micro plates [23]. Determination of the KHM value was carried out three times, then prepared each active fraction stock of 1000 µg/ml, positive control stock of chloramphenicol 1000 µg/ml, negative control of 20% DMSO solvent, growth control in the form of *Dickeya sp*. inoculum in MHB, and sterile MHB media control. Incubation was carried out for 18-24 hours at 37ºC. After incubation, turbidity value was measured with Hospitex plate reader at λ 600 nm to determine the KHM value.

After the turbidity value was measured, resazurin dye was added as much as 30 µl per hole as a qualitative test to determine the presence or absence of living bacterial cells. Observations and turbidity measurements were made again after 2 and 4 hours of incubation from resazurin staining. The KHM value was determined at the smallest test concentration value that showed the lowest turbidity value after 18-24 hours incubation and unchanged resazurin color after 2-4 hours.

Data Analysis

Data obtained from the results of this study were analysed descriptively by comparing the size of the inhibition zone, interpretation of the inhibition category, KHM value and turbidity. Data were presented in the form of pictures, graphs, and tables.

Phylogenetic Analysis

Isolation of genomic DNA from liquid culture of *Bacilus sp.* TSR 6 isolates with NB media were carried out based on the genomic DNA isolation method of Gram Positive Bacteria Wizard Genomic DNA Purification Kit Promega. Amplification of 16S rRNA gene using PCR with primers forward 63F (5'-CAGGCCTAACACATGCAAGTC-3') and reverse 1387R (5'-GGGCGGWGTGTACAAGGC-3') [24]. The PCR reagent used had a total volume of 25 µL including 10 µL of DNA template (15 ng/µL), 10.5 µL MyTaq HS Red Mix, 0.25 µL forward primer, and 0.25 µL reverse primer. PCR was performed with the following program: pre-denaturation at 95ºC for 5 minutes, followed by 30 cycles of denaturation at 95oC for 30 seconds, annealing at 52oC for 30 seconds, elongation at 72oC for 45 minutes, then final elongation at 72 ºC.

The amplification results were viewed through electrophoresis with the QIAxcel Advanced System, then sequencing was carried out using the Sanger method. Sequencing results were analyzed to find sequence similarities with those found in the gene bank database using the National Center for Biotechnology Information's Blast search program for nucleotides (BLASTN). Phylogenetic analysis was performed using MEGA 11 software [25]. All sequences were aligned using ClustalW multiple sequence alignment, phylogenetic trees were constructed using the Maximum Likelihood method and Hasegawa-Kishino-Yano model.

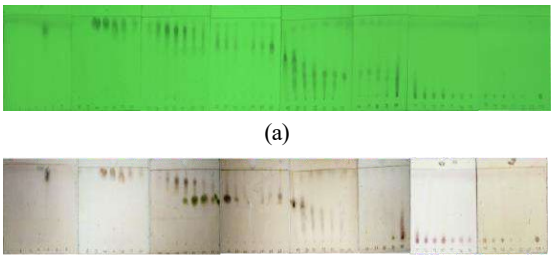
Results and Discussions

Production and Extraction of Antibacterial Compounds

Production of antibacterial compounds *Bacillus sp*. TSR 6 as much as 1 liter using liquid antibacterial production media. White colonies of *Bacillus sp*. TSR 6 bacterial isolates were seen growing on the surface of the production media starting from the third day to the fifth day of incubation time. Extraction of antibacterial compounds by partitioning with ethyl acetate solvent produced two fractions, namely the clear yellow ethyl acetate fraction at the top and the water fraction (concentrated filtrate) brownish at the bottom. The ethyl acetate fraction from the partition was obtained as much as 100 ml and produced a concentrated crude extract of 222 mg.

Separation of Antibacterial Compounds by Open Column Chromatography

Separation of candidate antibacterial compounds from 106 mg of crude extract of *Bacillus sp*. TSR 6 obtained 54 fractions (Figure 2). Based on the KLT results of the 54 fractions with 100% ethyl acetate eluent and reaction with cerium sulphate reagent, there are several fractions with the same stain separation pattern. Furthermore, fractions that have the same stain pattern are combined into 1 fraction so that 8 fractions of antibacterial compound candidates are obtained, namely: Fr. 1-9 (3 mg); Fr. 10-14 (2 mg); Fr. 15-17 (2 mg); Fr. 18-29 (19 mg); Fr. 30-38 (1 mg); Fr. 39-43 (13 mg); Fr. 44-52 (12 mg); and Fr. 54 (8 mg).



(a)

(b)

Figure 2. KLT results of fractions from open column chromatography. (a) UV 254 nm visualization; (b) reaction with cerium sulfate

The process of separating compounds through the elution process on the column will separate the compound components from the crude extract individually, allowing the separation of non-polar and polar compounds. More polar compounds will be absorbed strongly on the stationary phase and drop more slowly than non-polar compounds [22]. Based on this, the staining pattern formed in the KLT results of the separated initial fractions, namely Fr. 1-9; Fr. 15-17; and Fr. 18-29 may include non-polar compound components, then Fr. 30-38; Fr. 39-43, Fr. 44-52; and Fr. 54 may include polar compound components.

Screening of Antibacterial Activity by Agar Disc Diffusion

Based on the results of antibacterial activity testing of the 8 fractions of candidate antibacterial compounds, 3 fractions were found to be active in inhibiting *Dickeya sp*. namely Fr. 39-43; Fr. 44-52; and Fr. 54 presented in Table 1.

Table 1. Inhibition zone calculation of active fraction of candidate antibacterial compounds against *Dickeya sp*.

Sample	Repetition	Zone of inhibition (mm)		Average of Zone of inhibition (mm)
		1st Disk	2nd Disk	
Fr. 39-43	1	5	4	4,3
	2	5,5	2,5	
	3	4,5	4,5	
Fr. 44-52	1	4,5	10	5,2
	2	4,5	4	
	3	5	3	
Fr. 54	1	15	16	13,5
	2	14	11	
	3	13	11,5	
Positive Control (chloramphenicol)	1	20		20,8
	2	20		
	3	22		
Negative Control (MeOH)	1	-		-
	2	-		
	3	-		

Measurement of the average inhibition zone in Table 1 shows that the greatest antibacterial activity is found in Fr. 54 which is shown with an average inhibition zone diameter of 13.5 mm, while the other two fractions provide smaller antibacterial activity with an average inhibition zone of Fr. 44-52 by 5.2 mm and Fr. 39-43 by 4.3 mm. The positive control chloramphenicol gave an average inhibition zone diameter of 20.8 mm and the negative control did not give inhibition against *Dickeya sp*. indicating that the solvent used does not have antibacterial properties. Based on the inhibition category, it is known that Fr. 39-43 are classified as weak; Fr. 44-52 are classified as moderate; Fr. 54 is strong, and the positive control is very strong. The antibacterial activity of the three fractions in inhibiting *Dickeya sp*. characterized by the presence of an inhibition zone around the disc can be seen in Figure 3.

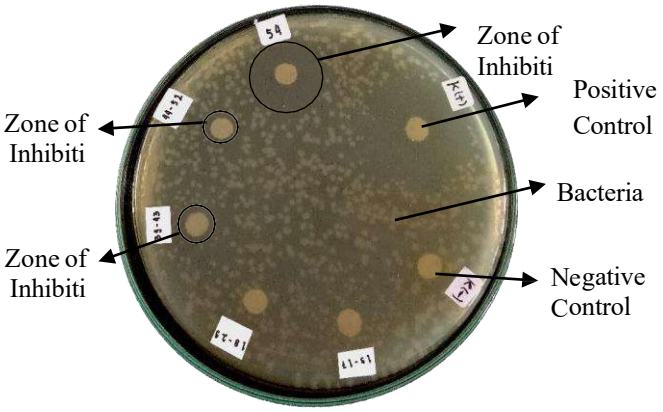


Figure 3. Antibacterial activity of the fraction resulting from the separation of compounds from the crude extract of *Bacillus sp*. TSR 6 against *Dickeya sp*.

Based on the inhibition category, the antibacterial compound activity contained in the active fraction against *Dickeya sp*. has inhibition that is classified as weak, moderate, and strong. However, the inhibition of the three active fractions is still not comparable to the positive control, chloramphenicol, even after compound separation. The inhibition of chloramphenicol is classified as very strong against *Dickeya sp*. The difference in inhibition indicates that antibacterial activity can be influenced by the type and character of each antibacterial compound, possibly related to the molecular size and diffusion power of the compound [26].

Preliminary Identification of Antibacterial Compounds in Active Fractions by KLT

The three active fractions were subjected to KLT with eluent n-Hex:ISOPROPANOL (7:3) and visualization under UV 254 nm, reaction with cerium sulfate and ninhydrin presented in Figure 4. Based on the results of KLT monitoring of the three active fractions under UV 254 nm and cerium sulfate reaction, there is a brownish stain that extends from Rf 0 to 0.5. The reaction with ninhydrin showed positive results, namely there was a purplish colored stain that extended from Rf 0 (bottom spot) to Rf 0.2. Furthermore, the difference in KLT results on the three active fractions with cerium sulfate reagent indicates that the compound components in each active fraction have compound components with different polarities. Fr. 54 is known to have the greatest antibacterial activity compared to Fr. 39-43 and Fr. 44-52.

The three fractions that showed different inhibition zones were possible due to differences in the polarity and molecular weight of each fraction. The Polarity and molecular weight of the compound affect the diameter of the inhibition zone formed. It is likely that Fr. 54 has high polar compounds [27]. This can be seen in the KLT results of Fr. 54 which shows a brownish stain only at Rf 0, meaning it has a very polar compound. The highly polar active compounds in Fr. 54 may be able to diffuse ideally in the agar medium so as to produce a large inhibition zone.

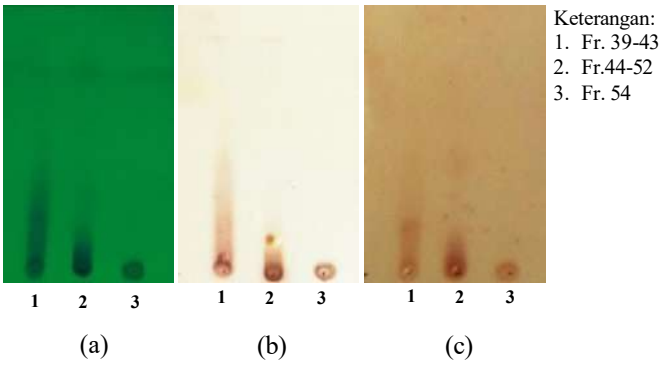


Figure 4. KLT results of the three active fractions capable of inhibiting *Dickeya sp*. UV visualization 254 nm; (b) cerium sulfate; (c) ninhydrin.

In addition, the results of KLT monitoring with ninhydrin reagent to identify lipopeptide compounds, showed positive results from the presence of purplish stains in each active fraction [15]. Lipopeptide compounds include amphiphatic molecules with non-polar groups in the form of fatty acid chains (hydrophobic) and polar in the form of

peptide bonds (hydrophilic). The presence of peptide bonds in the lipopeptide structure gives a positive reaction to ninhydrin [28].

The Rf value of the ninhydrin reaction results in each active fraction, Fr. 39-43 showed elongated purplish stains at Rf 0 to Rf 0.2, then Fr. 44-52 showed a slightly elongated purplish stain at Rf 0 to Rf 0.1, and Fr. The Rf value indicates that the three active fractions contain polar compound components with peptide bonds (Figure 4). This is in accordance with the KLT analysis of antibacterial compounds of previous studies that showed positive reaction results with ninhydrin, namely there is a purplish stain extending from Rf 0 indicating the presence of polar lipopeptide compounds [26]. Based on the results of the KLT analysis that gave positive results with ninhydrin, the compounds in the three active fractions are thought to contain peptide compounds. However, in this study it is not certain that the peptides contained in these compounds can bind to other compound molecules.

The activity of antibacterial compounds that are thought to come from the content of peptide compounds in the three active fractions may have a large molecular size so that their activity is limited to interacting with bacterial cell membranes. Based on previous research, it was reported that lipopeptide antibacterial compounds contained in the crude extract of *Bacillus* strain were able to inhibit the growth of *Dickeya* dadantii by targeting the destruction of bacterial cell membranes [5]. The lipopeptide compound group is known to have the ability to damage bacterial cell membranes by penetrating through hydrophobic interactions between fatty acids and hydrocarbon chains on membrane phospholipids resulting in the destabilization of the membrane structure. The altered membrane structure results in dehydration of the lipid head polar groups as well as changes in lipid constituents. This causes the formation of micelles from phospholipids, then pores are formed in the bilayer membrane so that membrane permeability is disrupted and leakage of cell contents occurs [29][30].

On the other hand, chloramphenicol is known to have a smaller molecular size so that it can easily diffuse and enter the cell. Chloramphenicol is known to target the 50S ribosomal subunit by suppressing the activity of the peptidyltransferase enzyme which results in inhibited protein synthesis. Based on this mechanism of action, chloramphenicol is known as a broad-spectrum antibacterial that can inhibit Gram-positive and Gram-negative bacteria [31].

Minimum Inhibitory Level (KHM) of Antibacterial Compound Fraction *Bacillus sp.* TSR 6 against *Dickeya sp.*

The results of KHM determination of the three active fractions against *Dickeya sp*. based on turbidity value readings and resazurin staining are presented in Table 2. KHM was only seen in Fr. 54 at a concentration of 500 µg/ml while the other two fractions did not show KHM in the concentration range tested. The positive control chloramphenicol had a KHM value of 15.6 µg/ml.

Table 2. KHM values of active fractions in inhibiting *Dickeya sp*.

Sample	KHM (µg/ml)
Fr. 39-43	-
Fr. 44-52	-
Fr. 54	500
Positive control (chloramphenicol)	15,6
Negative control (DMSO 20%)	-
Growth control	-
Sterile media control	-

When viewed from the zone of inhibition formed with a stock concentration of 5000 µg/ml, Fr. 39-43 and Fr. 44-52 are classified as having weak inhibition compared to Fr. 54 which is classified as strong against *Dickeya sp*. Antibacterial activity can be influenced by the concentration of antibacterial compounds, diffusion power, and the type of bacteria inhibited [26]. In addition, it may be influenced by the solubility of each compound in the solvent used. In determining KHM through microdilution, the solvent used was 20% DMSO, where the compounds Fr. 39-43 and Fr. 44-52 is not dissolved perfectly, it is related to the level of the polarity of each compound, so that its solubility in liquid media is not good and can affect its antibacterial activity [27].

The results of KHM determination of the three active fractions in inhibiting *Dickeya sp*. also measured the turbidity value after an incubation period of 18 hours at λ 600 nm. KHM value is seen from the lowest turbidity value. Turbidity value can be related to bacterial cell density [32]. Based on the measurement of turbidity values in Figure 5, the three fractions have the lowest turbidity value at a concentration of 500 µg/ml with an average turbidity value of 0.18.

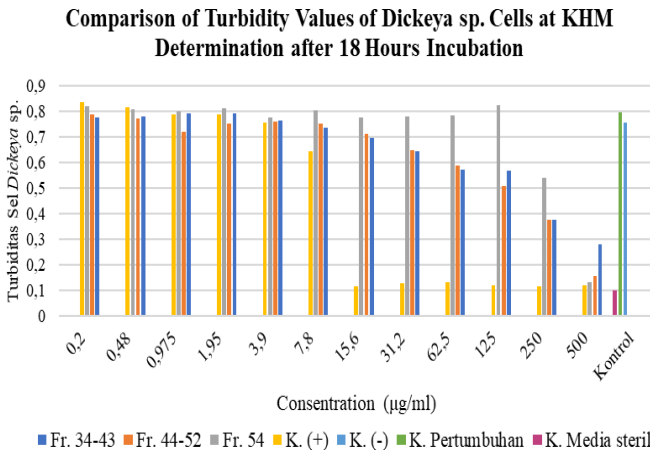


Figure 5. Comparison of turbidity values in KHM determination after 18 hours incubation.

It is known that Fr. 54 has a KHM value at a test concentration of 500 µg/ml which is indicated by the unchanging blue color of resazurin. Fr. 34-43 and Fr. 44-52 showed average turbidity values of 0.280 and 0.153 greater than the turbidity values of Fr. 54 and positive control. Whereas Fr. 54 gave the lowest turbidity value of 0.13. The positive control chloramphenicol also showed a low turbidity value of 0.117 at a concentration of 15.6 µg/ml.

Qualitative testing with resazurin staining (Figure 6) showed that Fr. 54 showed that the color of resazurin did not change at a concentration of 500 µg/ml after two hours of incubation time from the initial resazurin administration. The color change of resazurin was immediately seen at Fr. 39-43 and a slightly slower resazurin color change at Fr. 44-52. The color of resazurin from blue to pink indicates that there are still living bacterial cells, indicating that the test compound with the specified concentration has not been able to inhibit the test microbes completely. Living bacterial cells are able to reduce resazurin, which is blue and does not fluoresce, to fluorescent pink resofurin [23]. The results of resazurin staining in determining KHM can be seen in Figure 6.

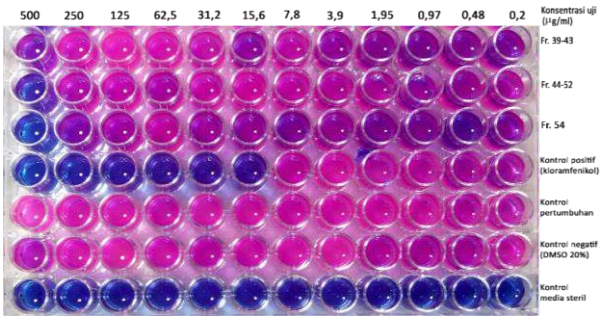


Figure 6. Determination of KHM of active fraction through microdilution with resazurin staining.

It is known that Fr. 54 has a KHM value at a test concentration of 500 µg/ml which is indicated by the unchanging blue color of resazurin. Fr. 34-43 and Fr. 44-52 showed a change in resazurin color at all test concentrations, indicating that there was no KHM in the concentration range tested and there was still bacterial cell growth. As a comparison, the positive control of chloramphenicol showed an unchanged resazurin blue color at the smallest concentration of 15.6 µg/ml, so it is known that the KHM of chloramphenicol is at that concentration. Negative control of 20% DMSO solvent and growth control showed a change in resazurin color to pink. Sterile MHB media control showed no change in resazurin color.

Based on the results of this study, it is known that *Bacillus sp*. TSR 6 bacteria have antibacterial compound activity against plant pathogenic bacteria *Dickeya sp*. The antibacterial compound activity is known to be in fraction 54. The results of KLT analysis of compounds in fraction 54 with ninhydrin reagent showed positive results indicating that the antibacterial activity of fraction 54 is likely due to the presence of compounds containing peptide bonds.

Phylogenetic Analysis of *Bacillus sp*. TSR 6 Isolate

The 16S rRNA gene sequencing results on *Bacillus sp*. TSR 6 isolate indicated that the isolate belongs to the genus *Bacillus* and has been identified as closely related to *Bacillus* vallismortis, *Bacillus sp*. TSR 6 isolate as strain RKB1 (Fig 7).

Based on phylogenetic analysis conducted in MEGA11, the evolutionary history was inferred by using the Maximum Likelihood method and Hasegawa-Kishino-Yano model [1]. The tree with the highest log likelihood (-2334.65) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with the superior log likelihood value. This analysis involved 32 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 1294 positions in the final dataset.

Bacillus vallismortis belongs to a group of *Bacillus* novel strains that were first discovered from the desert soil of Death Valley California [34]. Based on current related studies, it has been reported that B. vallismortis found in soil and some from healthy plant organs are also studied as biocontrol agents and have the potential to produce bioactive compounds such as surfactants and several types of lipopeptide compounds with activity to inhibit the growth of several phytopathogenic microbes [34][35][36][37][38]. The existence of this relatively new *Bacillus* strain with its potential to produce secondary metabolite compounds and have antibacterial activity, is expected to be able to add information related to antibacterial compounds produced by the *Bacillus* group from soil, especially compounds to inhibit plant pathogenic bacteria.

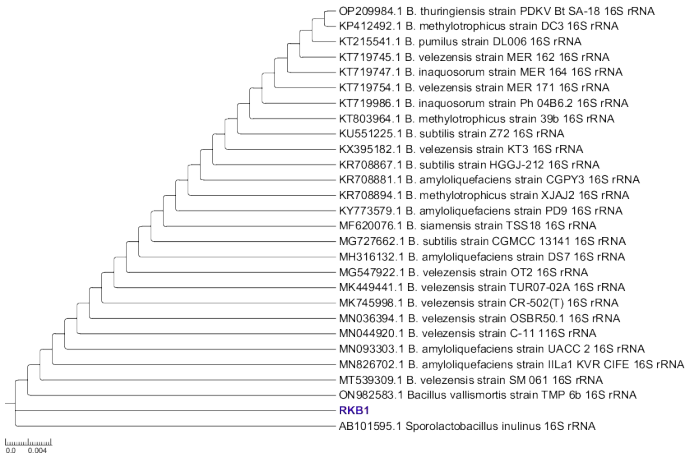


Figure 7. Phylogenetic tree of *Bacillus vallismortis* strain RKB 1 using the Maximum Likelihood method and Hasegawa-Kishino-Yano model with *Sprolacto Bacillus inulius* as outgroups.

Conclusions

Based on the results of this study, it is concluded that *Bacillus* vallismortis strain RKB1 from the soil of Kebun Raya Liwa (KRL) has the potential to produce antibacterial compounds against phytopathogenic bacteria *Dickeya sp*. The highest antibacterial compound activity of fractionation results was obtained through open column chromatography, namely fraction 54. The size of the inhibition zone of 13.5 mm is classified as strong inhibition with a KHM value of 500 µg/ml. The results of this study can be used as additional information related to the potential of *Bacillus* from soil in producing new secondary metabolite compounds, one of which is antibacterial which can be used as a biocontrol against plant pathogens. In addition, it is necessary to optimize *Bacillus* vallismortis RKB1 in the production of antibacterial compounds through modification of production media such as carbon sources, nitrogen, pH, and temperature so as to obtain antibacterial compounds with optimal concentrations and further compound analysis is carried out to find out information related to the structure, type, amount, and character of the compounds produced.

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