Isolation & Antagonistic Test Of Soil Bacteria Againstm *Rhizoctonia* Sp. A Pathogen Of Rice Plant (*Oryza Sativa* L.)

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Abstract

Purpose: This study aims to isolate bacteria from soil that are capable of producing antimicrobial compounds, as well as to test the inhibitory activity against the growth of *Rhizoctonia* sp.

Method: Soil samples were taken at 3 locations of PT Biotek Cipta Kreasi land, then cultured in nutrient agar media with stratified dilutions, then observations of colony morphology and cell morphology were made, then antagonistic tests were carried out in vitro and observations were made.

Results: The results showed that there were 23 isolates of soil bacteria with various characteristics and shapes. Then 17 gram-positive and rod-shaped bacteria were tested for antagonism, showing that the 17 bacteria did not have inhibition against the growth of *Rhizoctonia* sp.

Keywords: *Rhizoctonia* sp., isolation, soil bacteria, antagonistic test

Introduction

Microbial isolation is a way of separating these microbes from their natural environment and growing them as pure cultures in a growth medium. Before carrying out isolation, of course researchers must understand the growth requirements and ways to isolate it, as well as how to purify it. In nature, microbes are rarely found in a pure state, generally they are a mixture of various microbial species. Various ways to isolate and grow microbes are using spread plate, pour plate and streak plate method [1].

Antibiotic-producing microorganisms can be isolated from soil, seawater, mud, compost, rumen contents, domestic waste, rotten food, etc. Antibiotic-producing microbes are mostly obtained from soil microbes. The relationship between microorganisms in the soil can be neutral, competitive or even antagonistic. This antagonistic relationship is actually beneficial for human life, because with antagonism it is possible for one type of microorganism to produce anti-microbial substances which can inhibit the life of other types of microorganisms. Soil is a habitat for microorganisms, in one gram of soil there are millions of bacteria, fungi, protozoa and other microorganisms [2]. One of the bacteria that can produce antimicrobial compounds is the genus Bacillus. This group of bacteria is capable of producing antimicrobial compounds in the form of antibiotics, proteinases and bacteriocins [3]. Diseases in rice plants are very diverse, some of which are classified as important diseases because the resulting loss of yield is quite significant in affecting efforts to meet national rice production. These important types of disease are blast

disease by *Pyricularia oryzae*, bacterial leaf blight by *Xanthomonas oryzae* pv. oryzae, sheath blight by *Rhizoctonia solani*, brown spot by *Drehclera oryzae*, and bacterial grain rot by *Burkholderia glumae* [4]. Blast disease (*Pyricularia oryzae*) is one of the main diseases in rice which can cause losses of up to 61%[5]. Bacterial leaf blight can reduce rice production by 30-40%[6]. Meanwhile Nuryanto stated that yield losses due to sheath blight ranged from 20-35%[7].

One of the pathogens in rice plants is the fungus *Rhizoctonia* sp. which causes rice sheath blight. *Rhizoctonia* sp. is a soil-borne pathogen with a wide range of hosts in forestry and agricultural plant species. *Rhizoctonia* sp. is a fungus in the genus *Basidiomycetes* imperfect fungi[8]. *Rhizoctonia* sp. is a group of sterile fungi (cannot produce spores) but can produce sclerosia as a defensive structure both in the soil and in plant tissue[9].

Farmers' control of rice plant diseases is still dominated by the use of synthetic pesticides, while biological control is still very low. Biological control of rice plants is still limited because there are still few potential biological agents that can be developed as control technology. The limited types of biological agents are a challenge for conducting studies in order to obtain potential biological agents as candidates for developing biological control[4]. Continuous use of fungicides can result in dangerous impacts, so it is necessary to have environmentally friendly forms of control or biological control. One of the biological controls is the use of antagonistic bacteria made singly or in consortia[10].

So, the aim of this research is to isolate bacteria from soil that are capable of producing antimicrobial compounds, and to test their inhibitory activity on the growth of *Rhizoctonia* sp. fungal pathogens of rice plants.

Methods

This research employs an experimental method. This research was carried out in the research and development division at PT. Biotek Cipta Kreasi whose address is Kyai Samiyoredjo street, Jetis, Donolayan, Donoharjo, Ngaglik Subdistrict, Sleman Regency, Special Region of Yogyakarta.

Material and tools

The tools used are digital scales, spatula, aluminum foil, magnetic stirrer, measuring tube, laminar air flow (LAF), hot plate, vortex, petridish, autoclave, Erlenmeyer, test tube, test tube rack, Bunsen, measuring pipette, pipette pump , tube needle, object glass, cover glass, ziplock plastic, micropipette, micro tip, ruler, label and stationery as well as other tools that support this research. The materials used are soil, NA (Nutrient Agar) media, sterile Reverse Osmosis (RO) water, PDA (Potato Dextrose Agar) media, *Rhizoctonia* sp. isolate, 70% alcohol, 3% KOH, methylated spirits, gram dye.

Soil sampling

Soil sampling using a random method at the PT land location. Biotech Cipta Kreasi. Where there are 3 soil sampling locations, namely sample 1 was taken in the southern part of the RnD green house, sample 2 was taken in the chili land near the river and sample 3 was taken from the timun baby land. Soil samples were taken in the area around the plant roots in each field. Soil samples were taken using a spoon by digging the soil to a depth of 0 to 10 cm[11]. Take 1 tablespoon of each sample and

put it in a plastic ziplock.

Isolation and Purification

Isolate soil bacteria by weighing 1 gram of each soil sample. Then put it in a test tube containing 9 ml of sterile RO. Then it is vortexed to make it homogeneous, after which a multilevel dilution is carried out up to $1x10^4$. The resulting dilution was then placed in the oven at 80oC for 10 minutes. Then 0.5 ml was taken and inoculated in NA (Nutrient Agar) media in a petri dish. The petri dish is then covered in plastic wrap and incubated at room temperature. After the bacteria grow, purification is carried out by taking each bacterial colony and then growing it on NA petridish media using the quadrant streak technique. After 24 hours, single colonies were taken and purified by growing them in new NA petri media. After obtaining the pure culture, stock is made by growing it in NA Media so that it is tilted in a test tube. Each isolated bacterial isolate is given a name or code to make it easier to differentiate them[1].

Gram test

After obtaining the isolate from the isolation, it was continued with the KOH test by taking 1 dose of bacterial isolate and then mixing it evenly with 3% KOH which was placed on a glass object. Observations are made by looking at whether or not filaments such as mucus are produced.

Gram staining

Gram staining aims to identify bacteria including gram positive bacteria which are marked with a purple color or gram negative bacteria which are marked with a slightly red color. In this study, gram staining was carried out for selection of Bacillus sp. Gram staining is carried out by fixing the bacteria over a Bunsen flame using a physiological solution. The first stain was given a solution of crystal violet for 1 minute, iodine for 1 minute, sprayed with 70% ethanol, and safranin for 2 minutes, then the bacterial culture was observed under a microscope with 100× magnification[1].

Antagonist test

Fungal isolates need to be rejuvenated, so that the growth media is sufficient and can grow well. Rejuvenation of the fungus Rhizoctonia sp. This is done by taking 1 dose of fungal isolate and then growing it in media by scratching it and then incubating it. To determine the morphology of the fungus, the hyphae were taken, placed on a glass slide, then covered with a cover glass and observed with a microscope at 40x magnification. The selected bacterial isolates were then subjected to an antagonist test against Rhizoctonia sp, a pathogenic fungus on rice plants to see its inhibitory potential. The antagonist test was carried out using the dual culture method. The test was carried out by growing fungi together with soil bacterial isolates on PDA media in petri dishes. Rhizoctonia sp. which had previously been grown on PDA media in a petri dish was taken using a loop needle placed in the double culture test medium. Followed by adding soil bacterial isolates by inscribing them on the lines that have been made according to the Amal procedure (Figure 1)[12]. Then it is incubated and observed every day and the diameter of the pathogenic fungal colony is measured. The inhibitory activity of the fungus was seen whether the fungus passed the line or

not and then compared with the control.

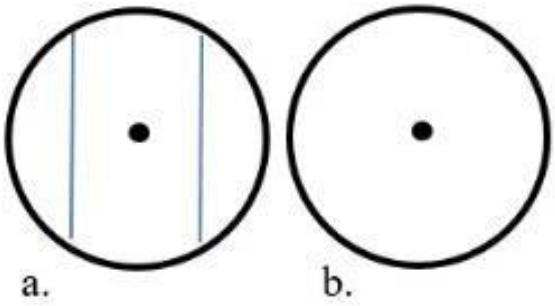


Figure 1. Simulation dual quture methode

Information:

- = *Rhizoctonia* sp.

= isolate soil bacterial

Observation

Observations were made by measuring the diameter of the fungus growing towards the bacteria (r2, vertical) and away from the bacteria (r1, horizontal). Then the percentage of resistance is calculated using the calculation formula[9]:
R= r1+r2/2
Inhibitory power presentation = R control - R treatment x 100%

Result

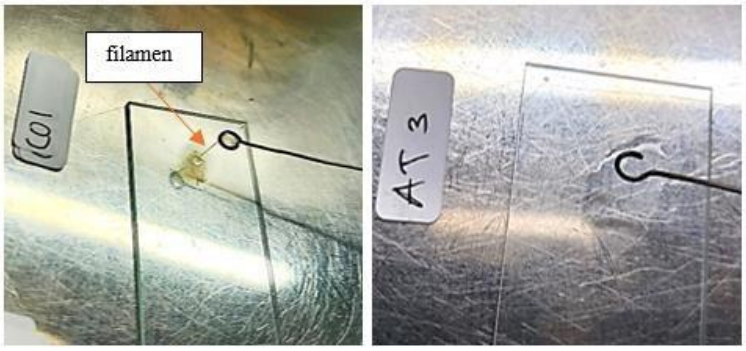
Isolation and purification of soil bacteria using multilevel dilutions then cultured using the spread technique, resulting in 23 single isolates that had been purified.

a b c

Figure 2. Isolation soil bacteria

a) soil sampel 1 (ST1); b) soil sampel 2 (ST2); c) soil sampel 3 (ST3)

Then purification is carried out by taking the growing bacterial colonies and growing them on Petri media using the quadrant streak method to obtain pure cultures with a single colony (Table 1). Then a gram test was carried out with 3% KOH which aims to detect gram negative bacteria. From the gram test with 3% KOH there were 3 gram negative bacteria, namely CT6, CO1, CD1 and CO3 (Figure 3a). The difference in the results of the 3% KOH test can be seen in (Figure 3).



a b

Figure 3. Gram test with KOH 3%

Table 1. Characteristics of Soil Bacterial Isolates

Bakteri	Origin	Color	Colony form	Cell shape	Gram Test (KOH 3%)	Gram staining
AB1	ST1	Transparent White	Small round	Streptobacil	-	+
AC2	ST1	White	Large round	Streptobacil	-	+
AT1	ST1	White	Small irregular	Streptobacil	-	+
AT3	ST1	Transparent White	Spindle	Streptobacil	-	+
AT4	ST1	Dull White	Small irreguler	Streptobacil	-	+
AT2	ST1	White	Spindle	Bacil	-	+
AC1	ST1	White	Spindle	Bacil	-	+
AB2	ST1	White	Small round	Bacil	-	+
BA1	ST2			Bacil	-	+
BA2	ST2	Sparkling white	Spindle	Bacil	-	+
BA3	ST2	White	Small rround	Bacil	-	+
BA4	ST2	Sparkling white	Round	Bacil	-	+
CT1	ST3	Orange	Round	Bacil	-	+
CT2	ST3	Putih	Round	Bacil	-	+
CT3	ST3	Putih	Large round	Bacil	-	+

CT4	ST3	Ivory White	Small round	Bacil	-	+
CT6	ST3	White	Small round	Bacil	+	-
CO2	ST3	White	Small round	Bacil	-	+
CT5	ST3	White	Filament	Cocus	-	+
CT7	ST3	Yellow	Round	Cocus	-	+
CD1	ST3	White	Irreguler	Cocus	+	-
CO1	ST3	Ivory White	Round	Cocus	+	-
CO3	ST3	White	Round	Cocus	+	-

From the results in Table 1, it is known that the 23 soil bacteria have various colors and shapes. Soil bacteria isolates are white, yellow and orange in color and their shapes are large round, small round, rooted, filamentous, irregular and spindle. From the results of the gram test, gram staining is then carried out to see the cell shape and gram color of the bacteria. Where the bacteria that will be tested as antagonists have long stem cells and chains. From the results of gram staining (Table 3), 5 isolates were found in streptobacil form, 12 isolates in bacillus form and 5 isolates in cocus form. These are gram positive bacteria except for 3 gram negative bacteria which produce thread filaments when tested with 3% KOH. So, after the gram staining test, 17 isolates were found with long rod-shaped characteristics and chains as well as gram-positive bacteria which were then tested as antagonists to the fungus *Rhizoctonia* sp.

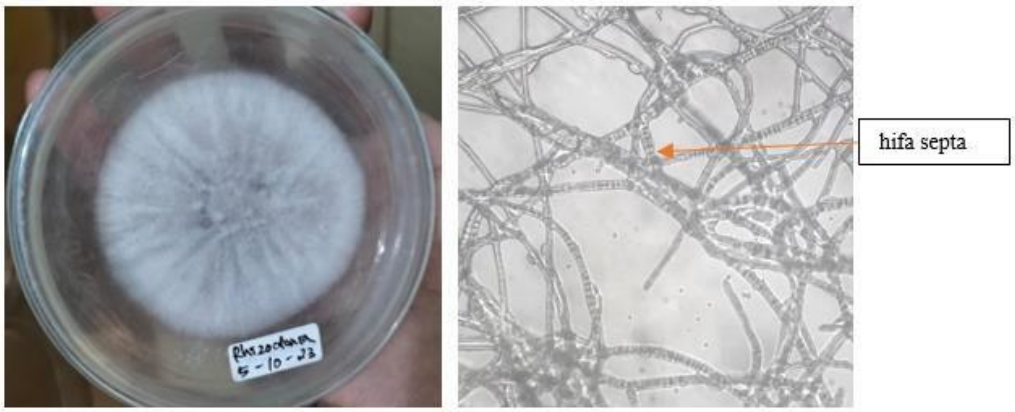


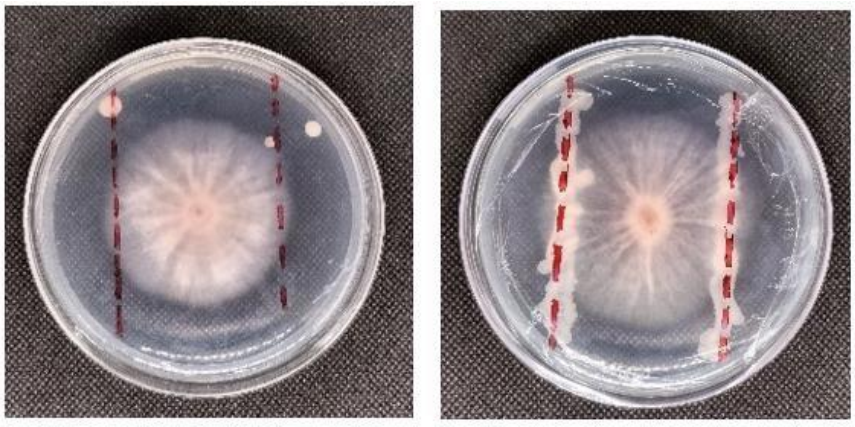
Figure 4. Isolats *Rhizoctonia* sp.

Rejuvenation of the fungus *Rhizoctonia* sp. carried out by culturing isolates of the fungus *Rhizoctonia* sp. on PDA media so that it is slanted 1 cycle. After the culture grows on the agar slant, it is then grown by inoculating it into PDA media in a petri dish (Figure 4). Then to see the fungal hyphae by observing under a microscope at the age of seven days using 40x magnification. The results of the rejuvenation, Rhizoctonia is white fungal isolates are brownish as they get older, have septa hyphae, do not have spores and have sclerotia (Figure 5).

Table 2. Antagonist Test of Soil Bacteria Againts *Rhizoctonia* sp.

Kode	Mean diameter Day 7 (mm)	Inhibitory power %
Control R	42,5	-
AB1	49	0
AC2	51	0
AT1	44	0
AT3	54	0
AT4	52	0
AT2	49,5	0
AC1	44	0
AB2	50	0
BA1	52	0
BA2	52	0
BA3	45	0
BA4	54,5	0
CT1	45	0
CT2	45	0
CT3	50	0
CT4	50	0
CO2	43	0

The 17 soil bacteria were tested for antagonism against *Rhizoctonia* sp. Antagonist test to determine the inhibitory power of soil bacteria on the growth of the pathogenic fungus *Rhizoctonia* sp. This inhibitory power can be seen from the difference in colony diameter of the control fungus *Rhizoctonia* sp. with the diameter of the fungal colony in the treatment of each bacteria. From the results of the soil bacterial antagonist test against *Rhizoctonia* sp. (Table 2) it can be seen that all soil bacterial isolates do not have an inhibitory effect on the growth of *Rhizoctonia* sp. Fungi grow past the line of bacterial growth. In (Table 2) the control *Rhizoctonia* sp. the average growth diameter is 42.5 mm. Meanwhile, all bacterial incision treatments did not produce an inhibition zone. With the average diameter of the fungus *Rhizoctonia* sp. more than 42.5 mm or beyond the bacterial growth line. So the inhibitory power of isolated soil bacteria is zero percent (0%).



a b

Figure 5. Antogonist test

(a) Control negative (b) Soil bacterial incision treatment.

Discussion

Research findings show bacterial colonies have different colors from one bacteria to another. Bacteria colors such as white, yellow, red, purple and so on. The shapes of bacterial colonies include circular (round, with edges), irregular, and rhizoid (like roots, spreading)[13]. Then a gram test was carried out with 3% KOH which aims to detect gram negative bacteria. Where gram-negative bacteria will produce thread-like filaments because they are in a high alkaline solution which causes the bacterial cell walls to rupture and produce thread-like filaments or mucus[14]. In gram staining, gram positive bacteria show a purple color or gram negative bacteria are characterized by a slightly red color[15].

Rhizoctonia sp. has a very wide range of host plants, apart from plants from the *Gramineae* family including cereals, namely corn, sorghum, wheat, grass and rice. This fungus also attacks plants from the *Leguminoceae* (legumes) family, *Solanaceae* and also the *Cucurbitaceae* family[16]. *Rhizoctonia* sp. has characteristics, namely, there is brown pigmentation of the hyphae, branching is formed in the distal septa of young cells as vegetative hyphae, narrowing of the hyphae occurs and septa are formed (Figure 5 b), the cytoplasm is connected through the pores of the septum (delipore)[17]. Multinucleated cells in young vegetative hyphae, there are no conidia, but there are monoloid cells, and there are no clamp connections and rhizomorphs, but there are sclerotia. These morphological characters have shown the characteristics of the fungus *Rhizoctonia* sp. although currently *Rhizoctonia* sp. have been further divided into groups of taxa based on the number of nuclei and their anastomosing ability[8].

In the result, soil bacterial isolates do not have an inhibitory effect on the growth of *Rhizoctonia* sp. Factors such as the production of antibacterial compounds, competition for nutrients, and complex microbial interactions can influence the effectiveness of soil bacterial inhibition in inhibiting the growth of certain pathogenic fungi[9]. Further isolation needs to be carried out with sampling locations in rice fields. And it is necessary to test the antagonists of bacterial isolates that have been isolated against other pathogens such as chili and cucumber plants because they

are suitable for the cultivated commodities in the soil of the sampling location. Also, further tests need to be carried out to identify bacterial isolates.

Conclusion

Based on the results of the research carried out, 23 isolates of soil bacteria were found with various characteristics and shapes. Then 17 gram-positive and rod-shaped bacteria were tested for antagonism, showing that the 17 bacteria did not have inhibition against the growth of Rhizoctonia sp. (0% inhibition power).

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