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Exploration and Characterization of Rhizobacteria from Rhizosphere of Rice Plants to Control *Fusarium fujikuroi* In Vitro

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Abstract

Rhizobacteria is bacteria that found in the root zone of plants. Rhizobacteria can act as biocontrol agents against *Fusarium fujikuroi*, the fungus that causes bakanae disease in rice plants. This study aimed to obtain rhizobacteria isolates from the roots of rice plants and to determine their characteristics as biocontrol agents against *F. fujikuroi* in vitro. This research consisted of two stages: 1) Exploration of rhizobacteria from the roots of rice plants in Padang City, West Sumatera, Indonesia and 2) Antagonistic character of Rhizobacteria against *F. fujikuroi in vitro* including: antagonistic test, hydrolytic enzyme production test (protease, amylase, and chitinase), and HCN compound production test. Based on the research, 32 rhizobacterial isolates were obtained as candidates for biological agents. All rhizobacterial isolates can suppress the growth of *Fusarium fujikuroi* in vitro with an inhibition percentage of 19.43-73.53%. The 4 best isolates in suppressing the growth of *Fusarium fujikuroi* are Kr 2.2 from Kuranji, KG 2.2 from Korong Gadang, PA 2.1 and PA 1.2 from Pasar Ambacang with an inhibition percentage of 60.39-73.53%. The four isolates are also able to produce hydrolysis enzymes (protease, amylase and chitinase) and HCN compounds.

Keywords: Antagonistic test, bakanae, biocontrol agents, HCN, hydrolysis enzymes

Introduction

Rice is the staple food for most Indonesian people (Winarko, 2023), around 5 tons/ha in 2021-2023 (Central Statistics Agency, 2024). This is lower when compared to its optimum productivity of 8 tons/ha (Aditya et al., 2021). One of the factors causing low productivity in Indonesia is the attack of bakanae disease, which is caused by the fungus *Fusarium fujikuroi* (Darnetty and Sulyanti, 2017).

F. fujikuroi can be a seed-borne or a soil-borne disease (Naeem et al., 2016). F. fujikuroi can infect seeds, resulting in failure to germinate, stunted, abnormal height growth, and yellowing leaves (Darnetty and Sulyanti, 2017). Advanced symptoms of bakanae disease are the plants turn brown and die, which is characterized by the presence of *F. fujikuroi* fungal hyphae at the base of the plant stem (Gupta et al., 2015). This is caused by secondary metabolites produced by *F.*

fujikuroi, namely gibberella hormones, fusaric acid, and fumonisins (Singh et al., 2023).

Bakanae disease has spread to Asian countries such as Japan, India, South Korea, and Indonesia (Shakeel et al., 2023). In Indonesia, rice plants in several areas have been reportedly attacked by bakanae disease. In West Sumatra, the highest attack rate (19.5-20%) was found in Padang City (Darnetty and Sulyanti, 2014). The IR 42 rice variety is a variety that is susceptible to the *F. fujikuroi* fungus, with an infection rate of 52.5% (Darnetty and Sulyanti, 2017).

Control of bakanae disease can be done preventively using resistant varieties, such as soaking seeds in hot water, burning or disposing of infected plant residues, and rotating crops. The control is carried out before the plants are infected. Control can be carried out for plants that have been infected using synthetic fungicides (Bashyal et al., 2022). Still, this control option has a negative impact on plants and the environment (Gurusinga et al., 2020). One recommended control is using biological agents from the rhizobacteria group (Jabiri et al., 2023).

Rhizobacteria are bacteria found in the rhizosphere, the surface of roots, or associated with roots (Kundan and Pant, 2015). Rhizobacteria colonize plant roots and interact with microorganisms in the rhizosphere, such as soil-borne pathogens (Abdelaziz et al., 2022). Rhizobacteria act as biological control agents against pathogenic fungi through direct mechanisms: antibiosis, competition, and lysis (Mardhiana et al., 2020). According to Umer et al. (2021), rhizobacteria produce antifungal secondary metabolites to inhibit the growth of pathogenic fungi.

Several species of rhizobacteria from the corn rhizosphere in Padang City, West Sumatra, Indonesia, such as *Bacillus cereus* AJ34, *Strenotrophomonas pavanii* LMTSA 5.4, and *Alcaligenes faecalis* AJ14, are able to control *Curvularia lunata* with an inhibition rate above 50% in vitro (Rahma et al., 2021). Rhizobacteria also produce hydrolytic enzymes: protease, amylase, and chitinase, that can lyse fungal cell walls (Fatima et al., 2022). Fatima et al. (2022) state that *Bacillus* sp. and *Micrococcus* sp. can produce HCN compounds that can inhibit the growth of pathogenic fungi. It is necessary to explore rhizobacteria in the soil of rice plant roots because rhizobacteria can act as biological agents on bakane disease. This study aimed to obtain rhizobacteria isolates from rice plant roots and determine their antagonistic ability as biological agents to control *F. fujikuroi* fungi in vitro.

Methods

The research was conducted in rice field of Padang City, West Sumatra, Indonesia, and the Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Padang, Indonesia, from Januari to June 2024.

Methods

This research consists of 2 stages: Exploration of rhizobacteria from rice plant roots, and antagonist character of rhizobacteria as biocontrol agents against *Fusarium fujikuroi* in vitro.

Exploration of Rhizobacteria

Rhizobacteria were isolated from the rhizosphere of IR 42 rice plants in six sub-districts in Padang City, West Sumatra, Indonesia: (A) Gunung Sarik (-0.9123685, 100.4164730), (B) Korong Gadang (-0.8954894, 100.4030238), (C) Limau Manis (-0.9318760, 100.3966327), (D) Pisang (0.9320832, 100.4428435), (E) Pasar Ambacang (-0.9384848, 100.4130851), and (F) Kuranji (-0.9154925, 100.4185568) (Figure 1). Samples were collected from 3 locations per sub-district. Soil samples were taken from

healthy rice plants aged 3–5 weeks at a 0–20 cm depth, weighing 20 grams per plant (Sudewi et al., 2021). Samples from the same field were combined and isolated in the laboratory.

Ten grams of soil were mixed with 100 ml of distilled water in a culture bottle, homogenized using a rotary shaker at 100 rpm for 30 minutes, then serially diluted to 10^{-6}. One mL of the final dilution was added to 9 ml of Nutrient Agar (NA) at 40°C, stirred, and poured into a petri dish using the pour plate method. After 48 hours of incubation, rhizobacteria colonies were isolated and streaked using the four-quadrant streak method to obtain pure colonies (Pudjiwati et al., 2019). Pure single colonies were stored in Eppendorf tubes containing 1 mL of sterile distilled water.

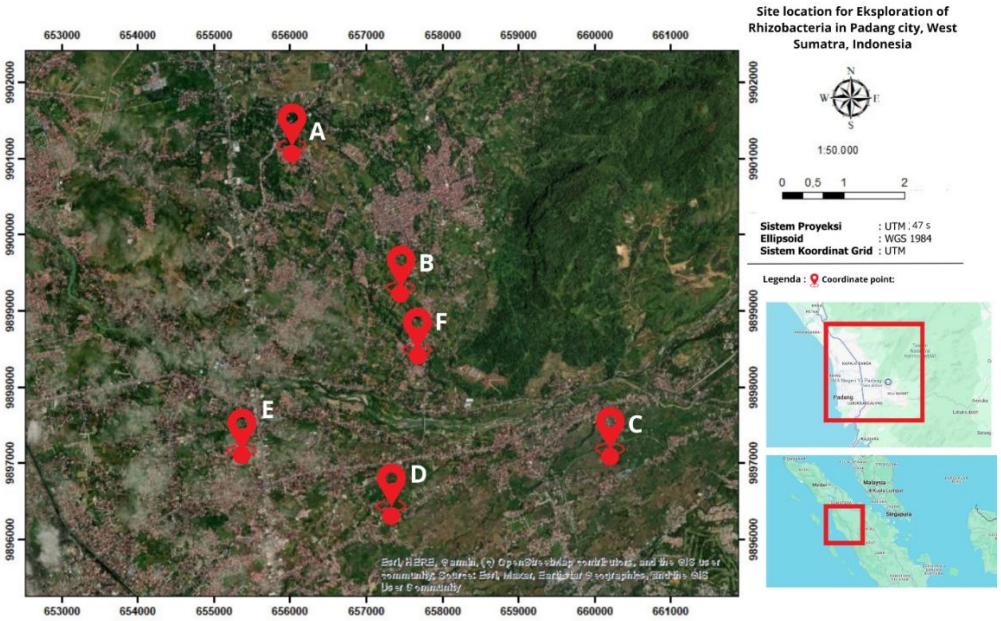


Figure 1. Site location for exploration rhizobacteria in Padang City, West Sumatra, Indonesia (A) Gunung Sarik, (B) Korong Gadang, (C) Limau Manis, (D) Pisang, (E) Pasar Ambacang, and (F) Kuranji

Gram Test

3% KOH was dropped on the object glass, then one colony of rhizobacteria was taken from the NA media and mixed with 3% KOH on the object glass (Hardiansyah et al., 2020).

Hypersensitivity Test

Testing for hypersensitive reactions aimed to determine the nature of rhizobacteria bacteria that are classified as pathogens to plants. Hypersensitive reactions are carried out by following the method used by Klement and Goodman (1967). All pure rhizobacteria isolates in petri dishes are suspended with sterile aquadest for 2x24 hours. The turbidity of the rhizobacteria suspension is compared with the McFarland solution on a scale of 8 (equivalent to a population density of 10^8 cells/mL) and infiltrated with a sterile 1 mL syringe without a needle on the lower surface of healthy tobacco plant leaves, covered with plastic and incubated for 2x24 hours.

Hemolysis Test

A hemolysis test was conducted using blood agar media (Sobieszczanska, 2007). One colony of rhizobacteria aged 2x24 hours was inoculated into the blood agar media using a sterile toothpick by dotting and incubating for 2x24 hours. Rhizobacteria, as candidates for

biological agents, do not produce clear zones in the blood agar media. Rhizobacteria that react positively will be eliminated.

Rejuvination of *Fusarium fujikuroi*

Rejuvenation of *F. fujikuroi* was carried out using a modified single-spore method (Hidayat, 2017). One cork-borer of *F. fujikuroi* was inserted into 5 mL of sterile aquadest and homogenized by vortexing so that the spores were released. The spore was observed under a microscope with up to 10 spores/field of view. The suspension was streaked in a zigzag manner on Water Agar (WA) (10 g of agar + 500 mL of aquadest). Germinated spores were grown on Potato Dextrose Agar (PDA) in a 9 cm diameter petri dish and incubated for 7 days at room temperature.

Antagonist Test

The antagonistic test of rhizobacteria against *F. fujikuroi* was carried out using the dual culture method (Figure 2) (Marcic et al., 2018). *F. fujikuroi* was taken using a 5 mm diameter coccus borer and grown on a mixture of NA and PDA media in a 9 cm petri dish. The distance between the edge of the petri dish and *F. fujikuroi* was 3 cm. The rhizobacterial isolate was grown on the same petri dish at a distance of 3 cm from *F. fujikuroi* by scratching it for 3 cm. The control petri dish did not use any rhizobacterial isolates, then incubated for 10 days.

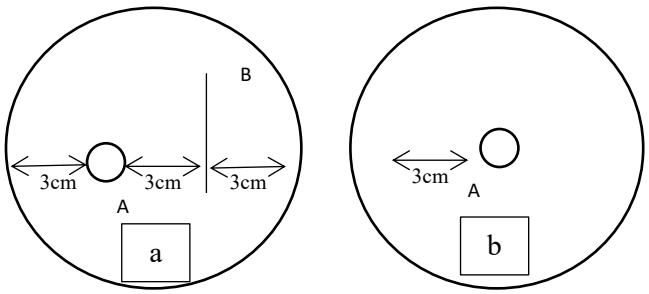


Figure 2. Sketch of antagonist test using dual culture method: (a) treatment, (b) control, (A) *F. fujikuroi*, (B) rhizobacteria

Protease Enzyme Production Test

Protease enzyme production test using skim milk + seawater complete agar (SWCA) media (5 g back to peptone; 0.1 g yeast extract; 0.3 mL glycerol; 75 mL sea water; 25 mL aqua dest; 1.5 g agar; and 1 g skim milk). Sterile filter paper with a diameter of 5 mm was dipped into a liquid culture of rhizobacteria in Nutrient Broth (NB) media. The filter paper was dried and placed on the SWCA media in a petri dish, dipped into sterile aquadest used as a control, and incubated for 2x24 hours (Sumardi et al., 2019).

Amylase Enzyme Production Test

Amylase enzyme production test using Starch Agar (SA) media (nutrient agar + 1% starch). Sterile filter paper with a diameter of 5 mm was dipped into a liquid culture of rhizobacteria in NB media, dried and placed on SA media. The filter paper was dipped into sterile distilled water used as a control and incubated for 2x24 hours. Then 2 mL of iodine solution was poured into the petri dish of the test rhizobacteria culture evenly, after which it was taken back with a sterile syringe (Wibowo et al., 2022).

Chitinase Enzyme Production Test

Chitinase enzyme production test using chitin agar media (2 grams of NA media; 2 g of colloidal chitin, 200 mL of aquadest). Sterile filter paper with a diameter of 5 mm was dipped into a liquid culture of rhizobacteria in NB media, dried, and placed on chitin agar media in a petri dish. The filter paper was dipped into a sterile aquadest used as a control and incubated for 5x24 hours (Suryadi et al., 2020).

HCN compound production test

The HCN compound production test from rhizobacteria was carried out based on the method developed by Bakker and Schipper (1987). Rhizobacteria were grown on NA media in a petri dish by scratching. The

center of the petri dish lid was attached with a sheet of filter paper that soaked in a picric acid solution (2 g of picric acid, 8 g of oodium carbonate, 200 mL of aquadest), incubated for 14 days (El-Rahman et al., 2019).

Observation

Morphology of rhizobacteria

The isolated rhizobacteria strains were characterized based on morphology such as pigmentation, shape, edge and elevation (Yadu et al., 2023).

Gram, hypersensitivity, and hemolysis test

Rhizobacteria are declared Gram-negative if the mucus is formed when the loop needle is removed from the 3% KOH mixture, and rhizobacteria are declared Gram-positive if the mucus is not formed (Hardiansyah et al., 2020). As a candidate for biological agents, Rhizobacteria should not cause necrosis symptoms in tobacco leaves (Schaad et al., 2001) and should not produce clear zones on blood agar media. Rhizobacteria that react positively are eliminated.

Percentage of inhibition

The inhibition by measuring the area of the *F. fujikuroi* fungal colony was calculated 10 days after inoculation using mm paper and entered into the growth inhibition by the formula (Niam, 2021):

$$\text{Inhibition } (\%) = \frac{\text{control}-\text{tested}}{\text{control}} \text{x } 100\% \quad \text{..... (1)}$$

Characteristics of rhizobacteria

Observation of enzyme production was carried out qualitatively by observing the clear zone formed around the rhizobacteria colony after 2x24 hours. Observation of rhizobacteria colonies on SWCA for protease enzymes (Sumardi et al., 2019), on SA for amylase enzymes (Wibowo et al., 2022), and on chitin agar for chitinase enzymes (Suryadi et al., 2020). The clear zone indicates a positive chitinase enzyme production test result. The chitinolytic index can be calculated using the formula:

$$\text{Indeks kitinolitik} = \frac{\text{D}-\text{d}}{\text{d}} \text{x } 100\% \quad \text{..... (2)}$$

Notes: D = total diameter of the clear zone and rhizobacterial colonies, d = diameter of rhizobacteria colony

Rhizobacteria ability to produce HCN was observed after 14x24 hours, indicated by a change in color on the filter paper to light brown (+), dark brown (++), and/or brick red (+++) (El-Rahman et al., 2019).

Data Analysis

The data percentage of inhibition of rhizobacteria against *F. fujikuroi* was statistically analyzed using Analysis of Variance (ANOVA). The analysis will continue with Duncan's test if it significantly differs at level 5%.

Results

Morphology of Rhizobacteria

Based on the results of isolation from the rhizosphere of rice plants in Padang City, 62 rhizobacteria isolates were obtained. The most isolates were obtained from Korong Gadang District (12 isolates), and the least were obtained from Pasar Ambacang District (8 isolates). Based on the color of the colony, generally, there was no pigmentation (40 isolates), circular shape (36 isolates), intact colony edges (35 isolates), and convex elevation angles (31 isolates) (Table 1).

Table 1. Morphology of rhizobacteria isolated from the soil of rice plant roots

No	Isolate code	Pigmentation	Shape	Margin	Elevation
1	Kr 1.1	Non-pigmentation	circular	entire	convex
2	Kr 1.2	pigmentation	irregular	undulate	umbonate
3	Kr 1.3	Non-pigmentation	irregular	undulate	convex
4	Kr 1.4	Non-pigmentation	rhizoid	rhizoid	flat

No	Isolate code	Pigmentation	Shape	Margin	Elevation
5	Kr 2.1	Non-pigmentation	circular	entire	convex
6	Kr 2.2	pigmentation	irregular	undulate	raised
7	Kr 2.3	Non-pigmentation	irregular	spiral	raised
8	Kr 3.1	Non-pigmentation	circular	entire	flat
9	Kr 3.2	Non-pigmentation	circular	entire	convex
10	Kr 3.3	Non-pigmentation	circular	entire	convex
11	Kr 3.4	Non-pigmentation	irregular	undulate	flat
12	KG 1.1	pigmentation	circular	entire	convex
13	KG 1.2	pigmentation	circular	entire	pulvinate
14	KG 1.3	Non-pigmentation	irregular	undulate	flat
15	KG 1.4	pigmentation	circular	entire	convex
16	KG 2.1	Non-pigmentation	filamentous	filamentous	flat
17	KG 2.2	pigmentation	circular	entire	convex
18	KG 2.3	pigmentation	circular	entire	umbonate
19	KG 2.4	Non-pigmentation	circular	entire	convex
20	KG 3.1	pigmentation	circular	entire	umbonate
21	KG 3.2	Non-pigmentation	circular	entire	convex
22	KG 3.3	pigmentation	circular	entire	convex
23	KG 3.4	Non-pigmentation	circular	entire	convex
24	GS 1.1	pigmentation	filamentous	filamentous	flat
25	GS 1.2	pigmentation	irregular	undulate	convex
26	GS 1.3	Non-pigmentation	irregular	undulate	flat
27	GS 1.4	pigmentation	circular	entire	convex
28	GS 2.1	Non-pigmentation	irregular	undulate	flat
29	GS 2.2	pigmentation	circular	entire	pulvinate
30	GS 2.3	pigmentation	circular	entire	pulvinate
31	GS 3.1	pigmentation	circular	entire	convex
32	GS 3.2	pigmentation	circular	entire	convex
33	GS 3.3	Non-pigmentation	irregular	undulate	flat
34	GS 3.4	pigmentation	circular	entire	convex
35	LM 1.1	Non-pigmentation	irregular	undulate	flat
36	LM 1.2	Non-pigmentation	circular	entire	convex
37	LM 1.3	Non-pigmentation	circular	entire	convex
38	LM 1.4	pigmentation	circular	entire	convex
39	LM 2.1	Non-pigmentation	irregular	spiral	umbonate
40	LM 2.2	Non-pigmentation	circular	entire	convex
41	LM 2.3	Non-pigmentation	circular	entire	convex
42	LM 2.4	Non-pigmentation	rhizoid	rhizoid	flat
43	LM 3.1	Non-pigmentation	irregular	spiral	convex
44	LM 3.2	Non-pigmentation	circular	entire	convex
45	LM 3.3	Non-pigmentation	filamentous	undulate	umbonate
46	Ps 1.1	pigmentation	circular	entire	convex
47	Ps 1.2	Non-pigmentation	irregular	undulate	flat
48	Ps 2.1	Non-pigmentation	circular	entire	convex
49	Ps 2.2	Non-pigmentation	circular	entire	convex

No	Isolate code	Pigmentation	Shape	Margin	Elevation
50	Ps 2.3	Non-pigmentation	circular	entire	convex
51	Ps 2.4	Non-pigmentation	irregular	undulate	flat
52	Ps 3.1	Non-pigmentation	irregular	undulate	flat
53	Ps 3.2	Non-pigmentation	irregular	undulate	flat
54	Ps 3.3	Non-pigmentation	irregular	undulate	flat
55	PA 1.1	Non-pigmentation	circular	entire	umbonate
56	PA 1.2	pigmentation	circular	entire	raised
57	PA 2.1	pigmentation	irregular	undulate	raised
58	PA 2.2	Non-pigmentation	circular	entire	convex
59	PA 2.3	Non-pigmentation	circular	undulate	convex
60	PA 3.1	Non-pigmentation	irregular	undulate	flat
61	PA 3.2	pigmentation	circular	entire	convex
62	PA 3.3	Non-pigmentation	irregular	spiral	flat

Gram, Hypersensitivity, and Hemolysis Test

There were 48 isolates of Gram-positive rhizobacteria and 14 isolates of Gram-negative. The selection of rhizobacteria with hypersensitivity test obtained 17 isolates as pathogens for plants, and 45 isolates were not pathogens for plants. The selection of rhizobacteria with a hemolysis test obtained 23 isolates as pathogens for mammals and 39 isolates were not pathogens for mammals. From the selection, 32 isolates were candidates for biological agents (Table 2). Candidate rhizobacteria as biological agents are used for testing in the next stage.

Table 2. Results of Gram test, hypersensitivity reaction and hemolysis test of rhizobacterial isolates

No	Isolate	Gram	Rx HR	Hemolysis test	Information
1	Kr 1.1	+	+	+	Non-candidate
2	Kr 1.2	+	-	-	Candidate
3	Kr 1.3	+	+	+	Non-candidate
4	Kr 1.4	+	-	+	Non-candidate
5	Kr 2.1	-	-	-	Candidate
6	Kr 2.2	+	-	-	Candidate
7	Kr 2.3	+	-	+	Non-candidate
8	Kr 3.1	+	-	-	Candidate
9	Kr 3.2	-	+	+	Non-candidate
10	Kr 3.3	+	-	-	Candidate
11	Kr 3.4	+	-	+	Non-candidate
12	KG 1.1	+	-	-	Candidate
13	KG 1.2	+	+	-	Non-candidate
14	KG 1.3	+	-	-	Candidate
15	KG 1.4	+	-	-	Candidate
16	KG 2.1	+	+	+	Non-candidate
17	KG 2.2	-	-	-	Candidate
18	KG 2.3	+	+	-	Non-candidate
19	KG 2.4	+	-	-	Candidate
20	KG 3.1	+	+	-	Non-candidate
21	KG 3.2	+	-	-	Candidate

No	Isolate	Gram	Rx HR	Hemolysis test	Information
22	KG 3.3	-	-	-	Candidate
23	KG 3.4	+	-	-	Candidate
24	GS 1.1	+	-	+	Non-candidate
25	GS 1.2	+	-	-	Candidate
26	GS 1.3	+	-	-	Candidate
27	GS 1.4	+	+	-	Non-candidate
28	GS 2.1	+	-	+	Non-candidate
29	GS 2.2	+	-	-	Candidate
30	GS 2.3	-	+	-	Non-candidate
31	GS 3.1	+	-	-	Candidate
32	GS 3.2	+	-	-	Candidate
33	GS 3.3	+	-	+	Non-candidate
34	GS 3.4	-	+	+	Non-candidate
35	LM 1.1	+	+	+	Non-candidate
36	LM 1.2	-	+	+	Non-candidate
37	LM 1.3	+	-	-	Candidate
38	LM 1.4	+	-	-	Candidate
39	LM 2.1	+	+	+	Non-candidate
40	LM 2.2	+	-	-	Candidate
41	LM 2.3	+	-	-	Candidate
42	LM 2.4	+	-	-	Candidate
43	LM 3.1	+	-	-	Candidate
44	LM 3.2	+	+	-	Non-candidate
45	LM 3.3	+	+	+	Non-candidate
46	Ps 1.1	-	-	-	Candidate
47	Ps 1.2	+	+	+	Non-candidate
48	Ps 2.1	-	-	-	Candidate
49	Ps 2.2	-	-	-	Candidate
50	Ps 2.3	-	-	+	Non-candidate
51	Ps 2.4	+	-	+	Non-candidate
52	Ps 3.1	+	-	-	Candidate
53	Ps 3.2	+	-	-	Candidate
54	Ps 3.3	+	-	+	Non-candidate
55	PA 1.1	-	+	-	Non-candidate
56	PA 1.2	+	-	-	Candidate
57	PA 2.1	+	-	-	Candidate
58	PA 2.2	-	-	-	Candidate
59	PA 2.3	+	-	+	Non-candidate
60	PA 3.1	+	-	+	Non-candidate
61	PA 3.2	-	-	+	Non-candidate
62	PA 3.3	+	-	+	Non-candidate

Percentage of Inhibition

Rhizobacteria showed ability in suppressing the growth of *F. fujikuroi*. Based on the antagonistic test, all rhizobacteria showed inhibitory ability, particularly Kr 2.2 and PA 2.1 (73.53%, and 71.24%) (Table 3).

Table 3. Inhibitory rate of rhizobacteria against *Fusarium fujikuroi*

No	Isolate code	Percentage of inhibition (%)	No	Isolate code	Percentage of inhibition (%)	No	Isolate code	Percentage of inhibition (%)
1	Kr 2.2	73.53 a	12	Ps 3.1	29.37 efg	23	KG 1.1	22.54 g
2	PA 2.1	71.24 a	13	GS 1.3	27.21 efg	24	Kr 3.1	22.42 g
3	KG 2.2	65.00 ab	14	GS 3.2	26.46 efg	25	Ps 3.2	22.25 g
4	PA 1.2	60.39 ab	15	KG 3.2	25.22 fg	26	GS 1.2	22.23 g
5	KG 3.4	53.06 bc	16	LM 2.4	25.20 fg	27	Kr 3.1	22.02 g
6	KG 2.4	51.86 bcd	17	Ps 2.1	25.13 fg	28	Kr 1.2	21.86 g
7	Ps 1.1	51.31 bcd	18	KG 3.3	23.92 fg	29	KG 1.4	21.29 g
8	LM 1.4	40.70 cde	19	LM 2.2	23.71 fg	30	Ps 2.2	21.27 g
9	PA 2.2	38.50 def	20	KG 1.3	23.67 fg	31	Kr 2.1	20.64 g
10	Kr 3.3	34.12 efg	21	GS 2.2	23.65 fg	32	LM 1.3	19.43 g
11	GS 3.1	31.42 efg	22	LM 2.3	23.54 fg	33	Control	0.000 h

Rhizobacteria inhibit the growth of *F. fujikuroi* by producing antifungal compounds, which are indicated by the presence of an inhibition zone between rhizobacteria and *F. fujikuroi*. The antifungal compounds produced cause *F. fujikuroi* to be unable to grow on the media. The difference in inhibitory rate between isolates is influenced by their ability to make various antifungal compounds. Rhizobacteria were also competitive with F. *fujikuroi* in antibiotic tests, where *F. fujikuroi* cannot grow past rhizobacteria. The hyphae that grow towards rhizobacteria become thinner (Figure 3).



Figure 3. Rhizobacterial antagonist test against *F. fujikuroi* aged 10 days: a. control, b. isolate Kr 2.2, c. isolate GS 3.1

Characteristics of Rhizobacteria

A total of eleven rhizobacterial isolates produced protease enzymes with a clear zone around the rhizobacterial colonies on SWCA media. Isolates that were able to produce protease enzymes were Kr 2.2, KG 2.2, PA 1.2, KG 2.4, Ps 1.1, LM 1.4, GS 3.1, Ps 3.1, Ps 2.1, LM 2.3 and LM 1.3. A total of eleven isolates produced amylase enzymes with a clear zone around the rhizobacterial colonies on SA media. Isolates that were able to produce amylase enzymes were Kr 2.2, PA 2.1, KG 2.2, PA 1.2, LM 1.4, PA 2.2, GS 3.2, KG 3.2, LM 2.4, Ps 2.1 and Kr 1.2. A total of ten rhizobacterial isolates produced chitinase enzymes with the presence of clear zones around the rhizobacterial colonies on chitin agar media. Isolates that were able to produce chitinase enzymes were Kr 2.2, Kr 3.3, KG 2.2, KG 2.4, KG 3.4, GS 1.1, LM 2.2, Ps 1.1, Ps 3.1 and PA 2.1 with a chitinolytic index of 1.18-1.32 mm (Table 4).

Table 4. Characteristics of rhizobacteria producing protease, amylase, chitinase and HCN compound enzymes.

No	Isolate code	Protease enzyme	Amylase enzyme	Chitinase enzyme index (mm)	HCN
1	Kr 2.2	+	+	1.29	+++
2	PA 2.1	-	+	1.25	+
3	KG 2.2	+	+	1.25	+++
4	PA 1.2	+	+	-	++
5	KG 3.4	-	-	1.25	-
6	KG 2.4	+	-	1.27	-
7	Ps 1.1	+	-	1.32	+
8	LM 1.4	+	+	-	+
9	PA 2.2	-	+	-	+
10	Kr 3.3	-	-	1.28	++
11	GS 3.1	+	-	1.24	-
12	Ps 3.1	+	-	1.28	-
13	GS 1.3	-	-	-	-
14	GS 3.2	-	+	-	-
15	KG 3.2	-	+	-	-
16	LM 2.4	-	+	-	-
17	Ps 2.1	+	+	-	+
18	KG 3.3	-	-	-	-
19	LM 2.2	-	-	1.18	+
20	KG 1.3	-	-	-	+
21	GS 2.2	-	-	-	-
22	LM 2.3	+	-	-	++
23	KG 1.1	-	-	-	-
24	Kr 3.1	-	-	-	-
25	Ps 3.2	-	-	-	-
26	GS 1.2	-	-	-	-
27	Kr 3.1	-	-	-	-
28	Kr 1.2	-	+	-	-
29	KG 1.4	-	-	-	-
30	Ps 2.2	-	-	-	-
31	Kr 2.1	+	-	-	-
32	LM 1.3	-	-	-	-
33	Control	-	-	-	-

As many as thirteen isolates of rhizobacteria produced HCN compounds with the color change of filter paper dipped in picric acid from yellow to brownish (Figure 4). Isolates that were able to produce HCN compounds were Kr 2.2, Kr 3.3, KG 2.2, KG 2.4, KG 3.4, GS 2.2, GS 3.1, LM 1.4, LM 2.2, LM 2.3, Ps 1.1, Ps 2.1 and PA 2.1 (Figure 4).

As many as thirteen isolates of rhizobacteria produced HCN compounds with

the color change of filter paper dipped in picric acid from yellow to brownish (Figure 5). Isolates that were able to produce HCN compounds were Kr 2.2, Kr 3.3, KG 2.2, KG 2.4, KG 3.4, GS 2.2, GS 3.1, LM 1.4, LM 2.2, LM 2.3, Ps 1.1, Ps 2.1 and PA 2.1 (Figure 5).

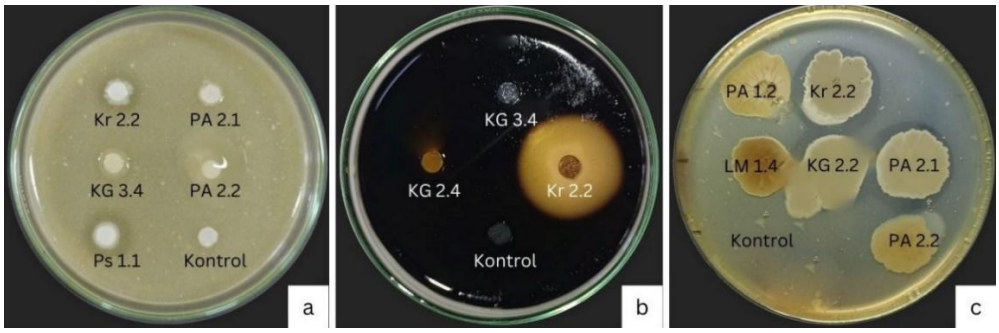


Figure 4. Characteristics of rhizobacteria as biocontrol agents: a. production of protease enzyme in SWCA media aged 2x24 hours, b. production of amylase enzyme in SA media aged 2x24 hours, c. production of chitinase enzyme in chitin agar media aged 5x24 hours

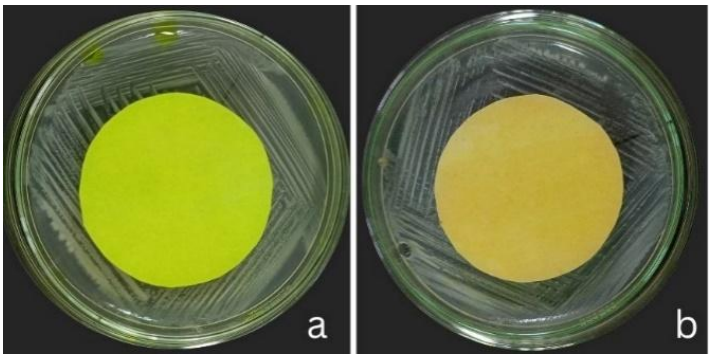


Figure 5. Production of HCN compound: a. isolate LM 1.4 has a negative HCN test, b. isolate PA 2.1 has a positive HCN test

Discussion

Based on the results, rhizobacterial isolates from rice plant roots in Padang City have diverse morphologies (Table 1). There are Gram-positive and Gram-negative rhizobacterial isolates (Table 2). This shows that rhizobacterial isolates also vary in genus and species. According to Tan et al. 2014, the reported rhizobacteria come from the genera Bacillus, Pseudomonas, Ochrobactrum, Lycinibacillus, Alcaligenes, Bradyrizobium, Rhizobium, Actinomycetes and Serratia. According to Maatoke & Oktovianus (2023), the types and diversity of rhizobacteria in one area differ from other regions, influenced by root exudates, soil environment, and interactions between soil microbes. Marfungah et al. (2023) stated that a well-maintained balance between soil, microbes, and plants can increase the population and diversity of rhizobacteria in the rhizosphere area.

Based on the hypersensitivity reaction and hemolysis tests, 32 rhizobacteria isolates were non-pathogenic to plants and mammals (Table 2). These isolates have the potential to be biological agents. The non-pathogenic nature of rhizobacteria in relation to plants and mammals is the main requirement in selecting biological agents. According to Pudjiwati et al. (2019), as biological agents, rhizobacteria must be safe for plants and mammals for food safety and health purposes.

Based on antibiotic tests, all rhizobacterial isolates were able to inhibit the growth

of *F. fujikuroi*. Isolates Kr 2.2 and PA 2.1 had the highest inhibition rate, 73.53%, and 71.24% (Table 3). Rhizobacteria inhibited the growth of *F. fujikuroi*, as indicated by an inhibition zone in the antibiotic test (Figure 3). The inhibition zone indicates that rhizobacteria produce antifungal compounds. Based on the antagonist test, rhizobacteria could also inhibit *F. fujikuroi* due to competition for nutrients and space. This is also supported by the characteristics of rhizobacteria, which can produce secondary metabolite compounds such as protease, amylase, chitinase, and HCN enzymes (Table 4). These compounds are produced by rhizobacteria when interacting with other microorganisms and are antimicrobial.

The production of lytic enzymes (protease, amylase, and chitinase) can cause hyphal malformation and hydrolyze fungal cell walls consisting of polysaccharides (Ariyani et al., 2021), starch (Prihatiningsih et al., 2017) and chitin (Yurnaliza et al., 2012). According to Agha et al. (2023), rhizobacteria isolates BCB16, BCB07, and BCB83 can inhibit the growth of *Fusarium oxysporum* >70% and produce secondary metabolites, including protease enzymes, cellulase, chitinase, siderophore, and HCN. According to Agustin et al. (2022), the difference in the inhibitory power of rhizobacteria isolates is influenced by the species and rhizobacteria antibiotics' ability to produce antimicrobial secondary metabolite compounds.

Based on Mohammed (2020), rhizobacteria from the genera Bacillus, Pseudomonas, Serratia, Aeromonas, Vibrio, and Enterobacter can produce chitinase enzymes and cause hydrolysis in several pathogenic fungi in plants. According to Rais et al. (2017), rhizobacteria from the *Bacillus* spp. group can produce protease enzymes and become biocontrol agents against *Pyricularia oryzae* in rice plants. Furthermore, rhizobacteria can also produce secondary metabolites: HCN compounds, that can inhibit the growth of *F. fujikuroi*. Based on Reetha et al. (2014), *Bacillus* spp. isolated from rhizobacteria from sunflower rhizosphere (*Helianthus annuus* L.) can also produce HCN. According to Tariq et al. (2017), rhizobacteria from the genera Bacillus, Burkholderia, Enterobacter, Herbaspirillum, Ochorobacteria, Pseudomonas, Serratia, Staphylococcus and Stenotrophomonas specifically destroy pathogenic fungal cells by producing antifungal compounds and bacteriocins.

Conclusion

Based on the research, 32 rhizobacterial isolates were obtained as candidates for biological agents. All rhizobacterial isolates can suppress the growth of *Fusarium fujikuroi* in vitro with an inhibition percentage of 19.43-73.53%. The 4 best isolates in suppressing the growth of *Fusarium fujikuroi* are Kr 2.2 from Kuranji, KG 2.2 from Korong Gadang, PA 2.1 and PA 1.2 from Pasar Ambacang with an inhibition percentage of 60.39-73.53%. The four isolates are also able to produce hydrolysis enzymes (protease, amylase and chitinase) and HCN compounds.

Declaration

Author contribution

Zola Puti Rahma is the first author for this paper. Haliatur Rahma is corresponding author and Eri Sulyanti is co-author. All authors read and approved the final paper.

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Competing interest

The authors declare that they have no known competing financial interests or

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References

Abdelaziz AM, AH Hashem, GS El-Sayyad, DA El-Wakil, S Selim, DHM Alkhalifah, MS Attia. 2022. Biocontrol of soil borne disease by plant growth promoting rhizobacteria. Tropical Plant Pathology 48(2): 1-23. DOI: 10.1007/s40858-022-00544-7

Aditya F, E Gusmayanti, J Sudrajat. 2021. Pengaruh Perubahan curah hujan terhadap produktivitas padi sawah di Kalimantan Barat. Jurnal Ilmu Lingkungan 19(2): 237-246. DOI: 10.14710/jil.19.2.237-246.

Agha SI, M Ullah, A Khan, N Jahan, SM Ullah, B Tabassum, S Parveen, Z Rehmat, A Hussain, S Ahmed, MH Hamdard. 2023. Biocontrol rhizobacteria enhances growth and yield of wheat (*Tricticumaestivum*) Under field condition againts *Fusarium oxysporum*. Bioenginered 14(1): 1-14. DOI: 10.1080/21655979.2023.2260923.

Agustin DA, AL Abadi, LQ Aini. 2022. Uji Mekanisme antagonis rizobakteri terhadap *Sclerotium rolfsii* penyebab rebah semai pada tanaman kacang tanah. Agropross: Nasional Conference Proceedings of Agricultur 296-307. DOI: 10.25047/agropross.2022.300.

Ariyani MD, TK Dewi, S Pujiyanto, A Suprihadi. 2021. Isolasi dan karakterisasi plant growth promoting rhizobacteria dari perakaran kelapa sawit pada lahan gambut. Bioma 23(2): 159-171. DOI: 10.14710/bioma.23.2.159-171.

Bakker AW, B Schippers. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biology and Biochemistry 19 (4): 451-457. DOI. 10.1016/0038-0717(87)90037-X

Bashyal BM, AK Gupta, P Parmar, J Yadav, R Choudhary, R Kumar, D Singh, R Aggarwal. 2022. Management of bakanae disease using fungicides and their effect on disease symptomatology. Indian Journal of Agricultural Sciences 92(9): 56-61. DOI: 10.56093/ijas.v92i9.112530.

Badan Pusat Statistik. 2024. Luas panen, produksi dan produktifitas padi menurut provinsi, 2021-2023. https://www.bps.go.id.

Darnetty, E Sulyanti. 2014. Distribusi dan Mating Populasi (MPs) fusarium yang berasosiasi dengan penyakit bakanae pada tanaman padi di Sumatera Barat. Seminar Nasional BKS PTN Barat. 768-773.

Darnetty, E Sulyanti. 2017. Respon beberapa varietas padi terhadap serangan *Fusarium fujikuroi* penyebab penyakit bakanae. JPT: Jurnal Proteksi Tanaman (Journal of Plant Protection 1(1): 18-23. DOI:10.25077/jpt.1.1.18-23.2017

El-Rahman AFA, HA Shaheen, RMA El-Aziz, DSS Ibrahim. 2019. Influence of hydrogen cyanide-producing rhizobacteria in controlling the crown gall and root-knot nematode, *Meloidogyne incognita*. Egyptian Journal of Biological Pest Control 29(41): 1-11. DOI: 10.1186/s41938-019-0143-7

Fatima I, S Hakim, A Imran, N Ahmad, M Imtiaz, H Ali, E Islam, S Yousaf, MS Mirza, F Mubeen. 2022. Exploring biocontrol and growth-promoting potential of multifaceted PGPR isolated from natural suppressive soil against the causal agent of chickpea wilt. Microbiological Research 1-13. DOI: 10.1016/j.micres.2022.127015

Gupta AK, IS Solanki, BM Bashyal, Y Singh, K Srivastava. 2015. Bakanae of rice an emerging disease in Asia. The Journal of Animal and Plant Sciences 25(6): 1499-1514. https://www.semanticscholar.org/Corpus ID: 53380605

Gurusinga RE, L Retnowati, S Wiyono, ET Tondok. 2020. Dampak penggunaan fungisida sintetik pada kelimpahan cendawan endofit tanaman padi. Jurnal Ilmu Pertanian Indonesia 25(3): 432-439. DOI: 10.18343/jipi.25.3.432

Hardiansyah MY, Y Musa, AM Jaya. 2020. Identifikasi plant growth rhizobacteria pada rizosfer bamboo duri dengan Gram KOH 3%.

Agrotechnology Research Journal 4(1): 41-46. DOI: 10.20961/agrotechresj.v4i1.40875

Hidayat I. 2016. Isolasi spora tunggal. Jurnal Mikologi Indonesia 1(1): 45-46. DOI: 10.46638/jmi.v1i1.13

Jabiri S, I Legrifi, M Benhammou, SE Laasli, F Mokrini, MB Amraoui, R Lahlali. 2023. Screening of rhizobacterial isolates from apple rhizosphere for their biocontrol and plant growth promotion activity. Aplied Microbiology 948-967. DOI: 10.3390/applmicrobiol3030065

Klement Z, RN Goodman. 1967. The hypersensitive reaction to infection by bacterial plant pathogens. Annual Review of Phytopathology 5: 17-44. DOI: 10.1146/annurev.py.05.090167.000313

Kundan R, G Pant, N Jadon, PK Agrawal. 2017. Plant Growth Promoting Rhizobacteria: Mechanism and current prospective. Journal of Fertilizers and Pesticides 6(2): 1-9. DOI: 10.4172/2471-2728.1000155

Maatoke CD, Oktovianus. 2023. Potensi rhizobakteri tanaman jahe (*Zhingiber officinale Linn. Var Rubrum*) di Kabupaten Halmahera Utara sebagai agen pemacu pertumbuhan tanaman. Bioscientist: Jurnal Ilmiah Biologi 11(1): 145-161. DOI: 10.33394/bioscientist.v11i1.7047

Marcic SM, V Todorovic, O Stanojovic, T Beric, S Stankovic, B Todorovic, I Potocnic. 2018. Antagonistic potential of *Bacillus* spp. isolates against bacterial pathogens of tomato and fungal pathogen of pepper. Pestic Phytomed 33(1): 9-18. DOI: 10.2298/PIF1801009M

Mardhiana, M Adiwena, AP Pradana. 2020. Karakterisasi bakteri dari perakaran *Nepenthes mirabilis* untuk pengendalian hayati *Fusarium oxysporum*. Buletin Penetian Tanaman Rempah dan Obat 31(1):48-58. http://repository.unej.ac.id/handle/123456789/104356

Marfungah S, F Puspita, B Tjahjono. 2023. Potetial of indigenous rhizobacteria as biocontrol agent of Xanthomonas sp.. Philippine Journal of Science 152(5): 1539-1548. DOI: 10.56899/152.05.02

Mohammed AF. 2020. Optimization of Cellulase and chitinase enzymes production by plant growth promoting rhizobacteria. Novel Research in Microbiology Journal 4(1): 641-652. DOI: 10.21608/nrmj.2020.73437

Naeem M, M Iqbal, N Parveen, SU Allh, Q Abbas, A Rehman, M Sad. 2016. An over view of bakanae disease of rice. American-Eurasian Journal of Agricculture and Environmental Science 16(2): 270-277. DOI: 10.5829/idosi.aejaes.2016.16.2.12834

Niam MY. 2021. Aktivitas antifungi isolat bakteri asal sarang rayap sebagai biokontrol fungi pathogen tanaman. https://eprints.walisongo.ac.id/id/eprint/16568/.

Prihatiningsih N, HA Djatmiko, P Lestari. 2017. Aktivitas siderofor *Bacillus subtilis* sebagai pemacu pertumbuhan dan pengendali .pathogen tanaman terung. Jurnal Hama dan Penyakit Tumbuhan Tropika 17(2): 170-178. DOI: 10.23960/j.hptt.217170-178

Pudjiwati EH, S Zahara, D Sartika. 2019. Isolasi dan karakterisasi yang berpotensi sebagai agen pemacu pertumbuhan tanaman. Jurnal Borneo Saintek 2(2): 1-10. DOI: 10.35334/borneo_saintek.v2i2.1084

Rahma H, Nurbailis, N Kristina. 2021. Plant Growth Promoting Rhizobacteria (PGPR): as a potential biocontrol for *Curvularia lunata* in vitro. Journal of Physics: Conference Series 1940: 012091. DOI: 10.1088/1742-6596/1940/1/012091

Rais A, Z Jabeen, F Shair, FY Hafeez, MN Hassan. 2017. *Bacillus* spp. a biocontrol agent enhances the activity of antioxidant defense enzymes in rice against *Pyricularia oryzae*. Plos One 1-17. DOI: 10.1371/journal.pone.0187412

Schaad NW, JB Jones, W Chun. 2001. Hipersensitivity Test. *Laboratory guide for identification of plant pathogenic bacteria*, Third edition. Plant Pathology 50(6): 812-814. DOI: 10.1046/j.1365-3059.2001.00635.x

Shakeel Q, M Mubeen, MA Sohail, S Ali, Y Iftikhar, RT Bajwa, MA Aqueel, SK Upadhyay, PK Divvela, L Zhou. 2023. An explanation of the mystifying bakanae disease narrative for

tomorrows rice. Microbiology 1-18. DOI: 10.3389/fmicb.2023.1153437

Singh V, A Kumar, P Kapoor, MS Bochalya. 2023. Detection of fumonisin among different strains of *Fusarium* spp. associated with bakanae disease of rice (*Oryza sativa* L.) using molecular markers. Enviromental Conservation Journal 24(4): 9-15. DOI: 10.36953/ECJ.15822520

Sobieszczanska BM. 2007. Hemolizyny *Escherichia coli*. Post Microbiology 46(4): 343-353. http://am-online.org/web/archiwum/vol4642007343.html

Sudewi S, B Patandjengi, AR Saleh, A Yani, Ratnawati. 2021. Eksplorasi rizobakteri penghasil giberelin dari padi lokal aromatic, Sulawesi Tengah. Seminar Nasional Politeknik Pertanian Negeri Pangkajene Kepulauan 310-316

Sumardi, S Farisi, CN Ekowati, MS Diana. 2019. Aktivitas dan karakterisasi enzim protease isolate *Bacillus* sp. (UJ132) secara kualitatif dan kuantitatif. Jurnal Riset Akuakultur 14(3): 193-199. http://ejournal-balitbang.kkp.go.id/index.php/jra

Suryadi Y, D Susilowati, IM Samudera, M Permatasari, L Ambarsari. 2020. Karakterisasi kitinase isolate bakteri rhizosfir asal Cianjur dan aktivitasnya terhadap pathogen *Colletotrichum* sp.. Bioma 9(1): 54-71. DOI: 10.26877/bioma.v9i1.6034

Tan KZ, O Radziah, MS Halimi, AR Khairuddin, SH Habib, ZH Shamsuddin. 2014. Isolation and characterization of rhizobia and Plant Growth Promoting Rhizobzcteria and their effect on rice seedling growth. American Journal of Agricultural and Biological Science 9(3): 342-360. DOI: 10.3844/ajabssp.2014.342.360

Tariq M, M Noman, T Ahmed, A Hameed, N Manzoor, M Zafar. 2017. Antagonistic features displayed by Plant Growth Promoting Rhizobacteria (PGPR) : a review. Journal of Plant Sciense and Phytopathology. 37-43. DOI: 10.29328/journal.jpsp.1001004

Umer M, M Mubeen, Y Iftikhar, MA Shad, HM Usman, MA Sohail, MN Atiq, A Abbas, M Ateeq. 2021. Role of rhizobacteria on plants growth and biological control of plant disease: A riview. Plant Protection 5(1): 59-73. DOI: 10.33804/pp.005.01.3565

Wibowo RH, W Darwis, Sipriyadi, M Adfa, E Silvia, R Wahyuni, DA Sari, Masrukhin. 2022. Bakteri penghasil amilase yang diisolasi dari ekoenzim limbah buah-buahan. Jurnal Biosilampari: Jurnal Biologi 4(2): 107-117. DOI: 10.31540/biosilampari.v4i2.1531

Winarko B. 2023. Karakteristik KTI komoditas padi global paling berpengaruh dan kontribusi Indonesia. Jurnal Perpustakaan Pertanian 32(2): 54-62. https://epublikasi.pertanian.go.id/berkala/jpp/article/view/3482

Yadu S, A Kerketta, RS Netam, K Thakur, A Singh, DK Pujari, N Yadav. 2023. Isolation and morphological characterization of indigenous rhizobacteria of Bastar Plateau. The Pharma Innovation Journal 12(10): 04-09. www.thepharmajournal.com

Yurnaliza, S Margino, L Sembiring. 2011. Kemampuan kitinase *Streptomyces* RKt5 sebagai antijamur terhadap pathogen *Fusarium oxysporum*. Jurnal Natur Indonesia 14(1): 42-46. DOI: 10.31258/jnat.14.1.42-46

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