

Bioprospecting of Essential Oil Nanoemulsion-Tolerant PGPR from Chili Rhizosphere as a Multifunctional Agent Against *Colletotrichum scovillei*

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ABSTRACT

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Plant Growth-Promoting Rhizobacteria (PGPR) offer a sustainable alternative to chemical fertilizers and pesticides in chili (*Capsicum annuum* L.) cultivation. This study aimed to explore and isolate potential PGPR from the chili rhizosphere in West Java Province using a novel selection method. Isolation was performed on Nutrient Agar (NA) medium supplemented with 2% biopesticide nanoemulsion, resulting in 80 successfully obtained isolates. Characterization results showed that 50 isolates were Gram-positive, 43 isolates exhibited positive hemolysis activity, and 43 isolates showed negative hypersensitivity reactions, indicating their potential safety for plant application. Bioassay screening identified 7 isolates (A2, A11, A12, B5.3, B5.5, B5.13, and C2.5) with strong antagonistic activity and 2 isolates (AT11, C5.2) capable of phosphate solubilization. Furthermore, enzymatic assays demonstrated significant functional diversity: 7 isolates produced chitinase, 9 produced amylase, and 14 produced protease. Specifically, several isolates exhibited multiple enzymatic and growth-promoting properties simultaneously. These findings suggest that indigenous PGPR from West Java, particularly those surviving on nanoemulsion-supplemented media, possess significant potential as biofertilizers and biocontrol agents to enhance chili productivity.

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1. Introduction

Red chili (*Capsicum annuum* L.) is one of the most strategic horticultural commodities in Indonesia, particularly in West Java Province, which serves as a major production hub. However, chili cultivation faces significant challenges; a primary factor for low productivity is the infestation of pathogens causing anthracnose disease, specifically fungi from the genus *Colletotrichum*. This disease can lead to yield losses of up to 100% and diminish fruit quality [1]. While farmers generally rely on chemical pesticides to manage anthracnose, this approach triggers various issues, such as pathogen resistance, environmental pollution, and adverse effects on human health [2]. Consequently, more sustainable and environmentally friendly control strategies are required [3]. One viable effort is the use of biological pesticides derived from essential oils [4] and the utilization of *Plant Growth-Promoting Rhizobacteria* (PGPR) as a biocontrol agent to manage pathogens in chili plants [5]. Furthermore, combining botanical pesticides with functional PGPR bacteria supports the principles of Integrated Pest Management (IPM) and holds the potential to reduce dependence on synthetic pesticides [6].

PGPR are bacteria that associate with plants in the rhizosphere, as well as within stem, leaf, and fruit tissues. They are capable of stimulating plant growth and suppressing pathogen development through both direct and indirect mechanisms [7]. Several studies have demonstrated that PGPR can inhibit *C. truncatum* by inducing systemic resistance in chili plants, characterized by a twofold increase in the production of phenylalanine ammonia-lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO), and β -1,3-glucanase compared to the control [8]. Furthermore, according to [9] PGPR establish beneficial relationships with plants by inhibiting the growth of *C. gloeosporioides*. PGPR have been shown to suppress anthracnose disease, resulting in a disease incidence of 19.33%, a disease severity of 16.56%, and an AUDPC value of 49.38 units [10].

The combination of two control techniques using PGPR and biopesticides offers a superior and integrated approach to managing anthracnose pathogens in chili. PGPR serves as a preventive component that strengthens plant resistance from within, while biopesticides provide direct protection against pathogens [11]. The use of biological pesticides is generally considered safe and highly compatible with functional PGPR due to their selective and biodegradable nature [12]. Unlike synthetic chemical pesticides, which often cause a drastic decline in soil microbial populations, biological pesticides such as those based on plant extracts or antagonistic fungi tend not to interfere with enzymatic activity or root colonization by bacteria such as *Pseudomonas fluorescens* or *Bacillus* spp. [13]. Furthermore, the integration of biological pesticides and PGPR frequently creates a synergistic effect, enhancing systemic plant resistance and maintaining the balance of the soil microbiota ecosystem to support sustainable agriculture.

Despite the significant potential of rhizosphere bacteria originating from chili plants in West Java, comprehensive screening that integrates biosafety assays (hemolysis and hypersensitivity tests) with functional enzymatic profiling remains limited. Therefore, this study aims to explore and isolate indigenous PGPR from West Java using media supplemented with nanoemulsions. These findings are expected to provide superior bacterial isolates capable of serving as multifunctional biofertilizers and bioprotective agents to support sustainable chili production.

2. Method

2.1. Sampling Procedures

Chili plant rhizosphere samples were collected from several chili farmlands across West Java Province. The soil adhering to the roots was sampled compositely at a depth of 10–20 cm. The samples were then placed in sterile plastic bags, stored in a cooling box, and immediately transported to the laboratory for the isolation process.

2.2. Supplemented Selective Media

Nutrient Agar (NA) was used as the base medium for isolation, supplemented with a botanical biopesticide nanoemulsion. The NA medium was prepared according to the manufacturer's instructions and sterilized using an autoclave at 121°C for 15 minutes. Once the medium temperature cooled to approximately 45–50°C, the biopesticide nanoemulsion was added at a concentration of 2%. The mixture was then poured into sterile Petri dishes and allowed to solidify.

2.3. Isolation and Purification of PGPR

Isolation was performed using the serial dilution method. A 10-gram soil sample was dissolved in 90 mL of physiological saline (0.85% NaCl) and shaken for 30 minutes. The suspension was diluted up to a factor of 10^3 . An aliquot of 0.1 mL from the last three dilutions was spread onto the surface of the media in Petri dishes and incubated at room temperature for 24–48 hours. The growing colonies were purified on new NA media until pure isolates were obtained. The diversity of PGPR was calculated using the Shannon-Wiener diversity index formula as follows:

$$H' = -\sum p_i \ln p_i$$

Where:

H' = Shannon-Wiener Diversity Index

π = Proportion of individuals of a particular species relative to the total population in the habitat

2.4. Gram Staining

The Gram status of the PGPR isolates was confirmed using the 3% KOH test. An isolate was identified as Gram-negative if a mucoid thread (slime) formed when the bacterial colony was mixed with 3% KOH, whereas it was identified as Gram-positive if no slime was observed [14].

2.5. Biosafety Assays

The hypersensitivity response (HR) test is considered positive if it shows symptoms of cell death, desiccation, and necrosis in the tobacco leaf area inoculated with the rhizobacteria suspension (Junita et al., 2024) Meanwhile, the hemolysis test is identified as positive if a clear zone (halo) forms around the rhizobacterial colonies on blood agar media [16]

2.6. Screening for Proteolytic Activity

Qualitative observation of protease enzyme production was conducted after 2x24 hours by observing the presence or absence of a clear zone formed around the PGPR colonies on SWCA media. The formation of a clear zone indicates a positive result for protease enzyme production [17]

2.7. Screening for Amylolytic Activity

Qualitative observation of amylase enzyme production was conducted after 2x24 hours by observing the presence or absence of a clear zone formed around the PGPR colonies on SA (Starch Agar) media. The formation of a clear zone indicates a positive result for amylase enzyme production (Manalu & Mariana, 2023)

2.8. Screening for Chitinolytic Activity

Qualitative observation of chitinase enzyme production was conducted by observing the presence or absence of a clear zone formed around the PGPR colonies on Chitin Agar media [19]. The formation of a clear zone indicates a positive result for chitinase production. The chitinolytic index can be calculated using the following formula

$$\text{Indeks kitinolitik} = \frac{D - d}{d} \times 100 \%$$

Where:

D = Total diameter of the clear zone and the PGPR colony (mm)

d = Diameter of the PGPR colony (mm)

2.9. Phosphate Solubilization Assay

Qualitative observation of the phosphate solubilization assay was conducted after 4x24 hours by observing the presence or absence of a clear zone formed around the PGPR colonies on Pikovskaya's agar media. The formation of a clear zone indicates a positive result for phosphate solubilization [20]. The Phosphate Solubilization Index (PSI) can be measured using the following formula

$$\text{Indeks fosfat} = \frac{D - d}{d} \times 100 \%$$

Where:

D = Total diameter of the clear zone and the PGPR colony (mm)

d = Diameter of the PGPR colony (mm)

2.10. Antagonistic Activity of PGPR against *Colletotrichum scovillei*

The experiment was conducted using a Completely Randomized Design (CRD), testing 37 PGPR isolates that showed negative results in both the hypersensitivity and hemolysis assays, along with one control. Each treatment was replicated three times. The antagonistic activity of the PGPR against *Colletotrichum scovillei* was evaluated using the dual culture method. 5 mm diameter mycelial plug of *C. scovillei* was taken using a cork borer and placed on PDA media in a 9 cm diameter Petri dish. The fungus was positioned 3 cm from the edge of the dish. The PGPR isolate was then inoculated on the same dish at a distance of 3 cm from *C. scovillei* by streaking it along a 3 cm line. The control plates contained only the fungal pathogen without any PGPR isolates. The cultures were incubated for 10 days. The observed parameter was the percentage of inhibition, determined by measuring the colony area of *C. scovillei*. The colony area was calculated on the 10th day post inoculation using millimeter (graph) paper and then integrated into the following growth inhibition formula

$$\text{Daya Hambat} = \frac{(\text{Luas Koloni Jamur Kontrol} - \text{Luas Koloni Jamur Uji})}{\text{Luas Koloni Jamur Kontrol}} \times 100\%$$

3. Results and Discussion

Screening and Isolation of Rhizobacteria on Nanoemulsion Media

The exploration of the chili rhizosphere in West Java using Nutrient Agar (NA) media supplemented with 2% biopesticide nanoemulsion successfully yielded a total of 80 pure isolates (Table 1). The incorporation of nanoemulsion into the isolation media functions as a unique selective agent. This ensures that the obtained isolates possess a high level of tolerance toward modern pesticide formulations a trait that is highly crucial if these bacteria are to be applied in intensive agricultural systems that utilize both chemical and nano-based biological inputs. According to [21], the success of PGPR application in intensive fields depends heavily on the isolates' ability to remain resilient and functional despite exposure to modern pesticide inputs; thus, selecting tolerant bacteria from the initial isolation stage is critical.

Morphological Characteristics and Biosafety Profiling of PGPR Isolates

Exploration was conducted across three distinct locations in West Java: Cikahuripan Village (isolates coded A), Leuwimalang Village (isolates coded B), and Ciherang Village (isolates coded C), successfully yielding a total of 80 isolates. Macroscopic observations revealed that the majority of the bacteria exhibited white colonies with circular shapes and entire margins. However, distinct color variations were observed, such as red in isolates A2 and A3, and cream in isolates B5.3 and B5.9. Diversity was also evident in colony elevation, which varied from raised and convex to flat and umbonate.

Table 1. Summary of PGPR Isolates, Morphological Characteristics, Gram Test, and Biosafety Assays

No	Isolate Code	Origin of Isolate	Character Morphology				Gram test	Hemolysis Test	Hypersensitivity Test (HR)
			Color	Forms	Margin	Elevation			
1	A 1	Cikahuripan Village	White	circular	entire	raised	-	-	-
2	A 2	Cikahuripan Village	Red	circular	entire	convex	+	-	-
3	A 3	Cikahuripan Village	Red	circular	entire	convex	+	-	-
4	A 4	Cikahuripan Village	White	irregular	undulate	raised	+	-	+
5	A 5	Cikahuripan Village	White	circular	entire	convex	-	-	+
6	A 6	Cikahuripan Village	White	circular	entire	convex	+	-	+
7	A 7	Cikahuripan Village	White	irregular	entire	raise	+	+	-

8	A 8	Cikahuripan Village	White	irregular	serate	flat	+	+	-
9	A 9	Cikahuripan Village	White	circular	entire	convex	+	-	+
10	A 10	Cikahuripan Village	White	circular	entire	convex	+	-	-
11	A 11	Cikahuripan Village	White	circular	entire	convex	+	-	-
12	A 12	Cikahuripan Village	White	circular	entire	umbonate	-	+	-
13	A 13	Cikahuripan Village	White	circular	entire	convex	-	-	-
14	A 14	Cikahuripan Village	White	circular	entire	convex	-	-	-
15	B 1.1	Leuwimalang Village	white	circular	entire	raised	-	+	-
16	B 1.2	Leuwimalang Village	white	irregular	entire	flat	-	+	-
17	B 2.1	Leuwimalang Village	white	circular	entire	umbonate	-	-	-
18	B 2.1	Leuwimalang Village	white	circular	entire	umbonate	-	-	-
19	B 2.3	Leuwimalang Village	white	circular	undulate	raised	-	+	-
20	B 2.4	Leuwimalang Village	white	irregular	undulate	flat	+	+	-
21	B 2.5	Leuwimalang Village	white	circular	undulate	convex	-	+	-
22	B 3.1	Leuwimalang Village	white	irregular	undulate	flat	-	+	-
23	B 3.2	Leuwimalang Village	white	irregular	serate	flat	+	+	-
24	B 3.3.1	Leuwimalang Village	white	circular	entire	convex	-	-	-
25	B 3.3.2	Leuwimalang Village	white	irregular	labote	raised	-	+	-
26	B 3.4	Leuwimalang Village	white	irregular	serate	flat	-	+	-
27	B 4.1	Leuwimalang Village	white	circular	entire	umbonate	-	+	-
28	B 4.2	Leuwimalang Village	white	circular	undulate	raised	-	-	-
29	B 4.3	Leuwimalang Village	white	circular	entire	umbonate	+	+	-
30	B 4.4	Leuwimalang Village	white	circular	entire	convex	-	-	-
31	B 4.5	Leuwimalang Village	white	circular	undulate	raised	+	+	-
32	B 5.1	Leuwimalang Village	white	circular	entire	convex	-	+	-
33	B 5.2	Leuwimalang Village	white	circular	entire	convex	-	-	-
34	B 5.3	Leuwimalang Village	cream	circular	entire	convex	-	-	-
35	B 5.4	Leuwimalang Village	white	irregular	serate	flat	-	+	-
36	B 5.5	Leuwimalang Village	white	circular	entire	convex	+	-	-
37	B 5.6	Leuwimalang Village	white	circular	undulate	raised	-	-	-
38	B 5.7	Leuwimalang Village	white	circular	entire	umbonate	-	-	-
39	B 5.8	Leuwimalang Village	white	irregular	entire	raised	+	+	-
40	B 5.9	Leuwimalang Village	cream	circular	entire	convex	-	-	-
41	B 5.10	Leuwimalang Village	white	circular	entire	umbonate	+	+	-
42	B 5.11	Leuwimalang Village	white	circular	entire	umbonate	-	-	-
43	B 5.12	Leuwimalang Village	white	irregular	entire	flat	+	+	-
44	B 5.13	Leuwimalang Village	white	circular	entire	convex	+	-	-
45	B 5.14	Leuwimalang Village	white	circular	entire	convex	-	-	-
46	B 5.15	Leuwimalang Village	white	irregular	entire	flat	+	+	-
47	B 5.16	Leuwimalang Village	white	circular	entire	convex	-	+	-
48	C 1.1	Ciherang Village	white	irregular	serate	flat	+	+	-
49	C 1.2	Ciherang Village	white	circular	entire	convex	+	+	-

50	C 1.3	Ciherang Village	white	circular	entire	convex	+	-	-
51	C 1.4.1	Ciherang Village	white	irregular	undulate	convex	+	-	-
52	C 1.4.2	Ciherang Village	white	irregular	undulate	raised	+	+	-
53	C 1.5.1	Ciherang Village	white	rhizoid	filamentous	raised	+	+	-
54	C 1.5.2	Ciherang Village	white	irregular	labote	raised	+	+	-
55	C 1.6.1	Ciherang Village	white	irregular	labote	raised	+	+	-
56	C 1.6.2	Ciherang Village	white	irregular	labote	convex	+	+	-
57	C 2.1	Ciherang Village	white	circular	entire	raised	+	+	-
58	C 2.2	Ciherang Village	white	circular	entire	raised	-	+	-
59	C 2.3.1	Ciherang Village	white	circular	entire	convex	+	+	-
60	C 2.3.2	Ciherang Village	white	irregular	labote	raised	+	+	-
61	C 2.4.1	Ciherang Village	white	irregular	labote	raised	+	+	-
62	C 2.4.2	Ciherang Village	white	circular	entire	convex	+	-	-
63	C 2.5	Ciherang Village	white	circular	entire	raised	+	+	-
64	C 2.6	Ciherang Village	white	circular	entire	raised	+	+	-
65	C 2.7	Ciherang Village	white	circular	entire	raised	-	-	-
66	C 2.8.1	Ciherang Village	white	circular	entire	convex	+	+	-
67	C 2.8.2	Ciherang Village	white	circular	entire	convex	+	+	-
68	C 3.1	Ciherang Village	white	circular	entire	raised	+	-	-
69	C 3.2	Ciherang Village	white	circular	entire	raised	+	-	+
70	C 4.1	Ciherang Village	white	irregular	labote	raised	+	+	-
71	C 4.2	Ciherang Village	white	circular	entire	raised	+	-	-
72	C 4.3A	Ciherang Village	white	circular	entire	raised	+	+	-
73	C 4.3B	Ciherang Village	white	circular	entire	flat	+	+	-
74	C 4.4	Ciherang Village	white	circular	entire	convex	+	+	-
75	C 4.5	Ciherang Village	white	circular	entire	convex	+	-	-
76	C 5.1	Ciherang Village	white	irregular	labote	raised	+	-	-
77	C 5.2	Ciherang Village	white	irregular	labote	raised	+	-	-
78	C 5.3.1	Ciherang Village	white	irregular	labote	flat	+	-	-
79	C 5.3.2	Ciherang Village	white	irregular	serate	flat	+	-	-
80	C 5.4	Ciherang Village	white	circular	entire	convex	+	+	-
	Positive						50	43	5
	Negative						30	37	75

In the biochemical and biosafety characterization, the Gram test using KOH 3% revealed that the majority of the bacterial population (50 isolates) were Gram-negative, while the remaining 30 isolates were Gram-positive. Furthermore, the hemolysis test was conducted to assess potential pathogenicity toward mammals, resulting in 43 positive isolates and 37 negative isolates. According to Amaria et al. (2023), the Hypersensitivity Response (HR) and hemolytic activity are critical factors in screening safe isolates to ensure they are not pathogenic to plants or other organisms. Based on the data, the majority of the isolates (75 isolates) exhibited a negative HR, meaning they do not cause necrosis in plant tissues and are potentially safe for use as plant growth-promoting agents. Only 5 isolates (A4, A5, A6, A9, and C3.2) showed a positive HR; consequently, these isolates were eliminated from the PGPR candidate list due to their risk of being phytopathogenic.

Rhizobacterial Diversity and Distribution Across Locations

Based on the analysis of PGPR microbial diversity across the three study sites, the biodiversity levels were consistently classified within the moderate category ($1.0 < H' < 3.0$). Data revealed that Leuwimalang Village reached the highest Shannon-Wiener Diversity Index (H') at 2.1, followed by Ciherang Village at 1.9, and Cikahuripan Village with the lowest value of 1.6. Despite these numerical variations, all locations maintain a moderate diversity classification. This moderate H' value indicates a relatively stable microbial community with an even distribution of individuals among isolates and no extreme dominance by a single species, which is essential for maintaining the ecological balance of the chili rhizosphere.

Table 2. Diversity Indices of Rhizobacterial Isolates Across Different Locations

Location	index Shannon-Wiener diversity	Category
Cikahuripan Village	1.6	Diversity currently
Leuwimalang Village	2.1	Diversity currently
Ciherang Village	1.9	Diversity currently

The moderate level of diversity indices indicates that the soil ecosystems in Cikahuripan, Leuwimalang, and Ciherang villages possess relatively stable bacterial community structures and adequate environmental productivity. The higher index in Leuwimalang compared to other locations suggests a better balance between species richness and evenness within the PGPR population in that area. Conversely, the lower value in Cikahuripan Village may be influenced by more homogeneous environmental factors or specific ecological pressures that limit the growth of certain species. According to [22], environmental factors that constrain ecological niches are proven to suppress the dominance of specific species through complex biotic and abiotic filtering mechanisms. In this context, the use of nanoemulsion-selective media also acts as an abiotic filter that determines which isolates can survive and establish the observed community structure.

Functional Potential of PGPR: Enzymatic Activity and Biofertilizer Properties

Functional screening of the 37 selected isolates revealed significant variations in enzymatic activities and phosphate solubilization. The data showed that 14 isolates produced protease, 9 produced amylase, and 7 exhibited chitinolytic activity. However, hydrogen cyanide (HCN) production was not detected in any of the tested isolates. Regarding biofertilizer characterization, only isolate C 5.2 from Ciherang Village demonstrated phosphate-solubilizing capabilities. Accumulatively, isolates from Cikahuripan Village exhibited a higher frequency of enzymatic activities compared to other locations, while isolates from Leuwimalang Village mostly yielded negative results across all parameters (Table 3). The limited enzymatic activity in certain isolates suggests ecological niche specialization or differing metabolic requirements within their original rhizosphere environments.

Table 3. Functional Characterization of Isolates: PGPR Potential, Enzymatic Activity, and Biofertilizer Properties

No	Isolate Code	Origin of Isolate	Characterization of PGPR as a biocontrol agent				Characterization of PGPR as a biofertilizer agent (phosphate solubilizer)
			Production of protease enzymes	Production enzyme amylase	Production enzyme chitinase	Production compound hydrogen cyanide (HCN)	
1	A 1	Cikahuripan Village	+	-	+	-	-
2	A 2	Cikahuripan Village	+	-	-	-	-

3	A 3	Cikahuripan Village	+	+	+	-	-
4	A 4	Cikahuripan Village	-	+	+	-	-
5	A 5	Cikahuripan Village	+	-	-	-	-
6	A 6	Cikahuripan Village	-	-	+	-	-
7	A 9	Cikahuripan Village	-	-	+	-	-
8	A 10	Cikahuripan Village	-	-	-	-	-
9	A 11	Cikahuripan Village	+	+	-	-	-
10	A 13	Cikahuripan Village	+	-	-	-	-
11	A 14	Cikahuripan Village	+	+	-	-	-
12	B 2.1.1	Leuwimalang Village	-	-	-	-	-
13	B 2.1	Leuwimalang Village	-	-	-	-	-
14	B 3.3.1	Leuwimalang Village	-	-	-	-	-
15	B 4.2	Leuwimalang Village	-	-	-	-	-
16	B 4.4	Leuwimalang Village	-	-	-	-	-
17	B 5.2	Leuwimalang Village	-	-	-	-	-
18	B 5.3	Leuwimalang Village	+	-	-	-	-
19	B 5.5	Leuwimalang Village	+	-	-	-	-
20	B 5.6	Leuwimalang Village	-	+	-	-	-
21	B 5.7	Leuwimalang Village	-	-	-	-	-
22	B 5.9	Leuwimalang Village	-	-	-	-	-
23	B 5.11	Leuwimalang Village	-	-	-	-	-
24	B 5.13	Leuwimalang Village	+	-	-	-	-
25	B 5.14	Leuwimalang Village	-	-	-	-	-
26	C 1.3	Ciherang Village	+	+	-	-	-
27	C 1.4.1	Ciherang Village	+	+	-	-	-
28	C 2.4.2	Ciherang Village	-	-	-	-	-
29	C 2.7	Ciherang Village	-	-	-	-	-
30	C 3.1	Ciherang Village	-	-	-	-	-
31	C 3.2	Ciherang Village	-	+	-	-	-
32	C 4.2	Ciherang Village	-	-	-	-	-
33	C 4.5	Ciherang Village	-	-	-	-	-
34	C 5.1	Ciherang Village	+	+	+	-	-
35	C 5.2	Ciherang Village	+	-	+	-	+
36	C 5.3.1	Ciherang Village	-	-	-	-	-
37	C 5.3.2	Ciherang Village	-	-	-	-	-
38	Control		-	-	-	-	-
Positive			14	9	7	0	1
Negative			24	29	31	38	37

The capability of isolates to produce protease, amylase, and chitinase enzymes highlights their significant potential as biocontrol agents. The production of chitinase and protease is a crucial antagonistic mechanism, as these enzymes synergistically degrade the primary structural components of fungal pathogen cell walls, which are composed of chitin and proteins. Isolates A 3, C 5.1, and C 5.2 are particularly notable for possessing multiple enzymatic activities simultaneously. This multi-enzymatic profile suggests the presence of dual or multiple defense mechanisms, which offer more robust suppression of pathogens in the field. Conversely, the absence of HCN production in all isolates indicates that the biocontrol potential of the tested bacteria does not rely on toxic volatile compounds but is predominantly driven by enzymatic pathways. This aligns with [23], who stated that antagonistic activities lacking HCN production direct the defense mechanism toward lysis via extracellular hydrolytic enzymes rather than volatile-mediated inhibition. Furthermore, the lack of HCN production presents an environmental safety advantage, as it minimizes the risk of toxic volatile residues affecting non-target organisms within the rhizosphere.

Regarding its role as a biofertilizer, the rarity of phosphate-solubilizing isolates found only in isolate C 5.2 demonstrates that the ability to solubilize bound phosphate in the soil is a highly specific trait not shared by the entire rhizobacterial population at the study sites. Isolate C 5.2 emerges as the

most promising candidate due to its unique multifunctional characteristics, acting as both a biocontrol agent through protease and chitinase production and a biofertilizer agent via phosphate solubilization. According to [21] such multifunctional traits are highly advantageous in the development of commercial bioinoculants. Isolates with dual mechanisms can support plant growth comprehensively by providing active protection against diseases (biotic pathway) while simultaneously enhancing the availability of critical nutrients in the soil (abiotic pathway). This competitive advantage renders isolate C 5.2 a superior candidate for application in sustainable agricultural systems.

Quantitative Analysis of Chitinolytic and Phosphate Solubilization Indices

Based on the assays performed on seven isolates with positive chitinase reactions, significant variations were observed in their chitin degradation and phosphate solubilization capabilities. Isolates from Cikahuripan Village exhibited stable chitinolytic activity with indices ranging from 1.3 to 3.0. In contrast, isolates from Ciherang Village showed more diverse characteristics; isolate C 5.1 had the lowest chitinolytic index at 0.8, while isolate C5.2 demonstrated dual activity with a chitinolytic index of 2.8 and a phosphate solubilization index of 0.68. Isolate A 4 was identified as having the most superior chitinolytic activity among all tested isolates (Table 4). The high chitinolytic index of isolate A 4 indicates an efficient extracellular enzyme system in hydrolyzing chitin polymers. Although isolate A 4 excels in chitinolytic activity, isolate C 5.2 offers a strategic advantage as a multifunctional candidate due to its dual role as both a biocontrol agent and a nutrient solubilizer.

Table 4. Evaluation of Chitinolytic and Phosphate Solubilizing Indices among Potential PGPR Isolates

No	Isolate Code	Origin of isolate	index chitinilytic	Index Phosphate
1	A1	Cikahuripan Village	2.2	-
2	A 3	Cikahuripan Village	2.7	-
3	A 4	Cikahuripan Village	3.0	-
4	A6	Cikahuripan Village	1.3	-
5	A9	Cikahuripan Village	2.2	-
6	C.5.1	Ciherang Village	0.8	-
7	C.5.2	Ciherang Village	2.8	0.68

The high chitinolytic indices observed in isolates A 4 (3.0) and C 5.2 (2.8) indicate their significant potential to produce effective chitinase enzymes for degrading chitin polymers. This capability is frequently associated with the potential of these isolates as biocontrol agents against pathogens possessing chitin-based cell walls [24]. Interestingly, isolate C 5.2 emerged as the only isolate in this study with dual functional capabilities: chitin degradation and phosphate solubilization. The presence of a phosphate solubilization index in C 5.2 demonstrates its ability to convert bound phosphate into plant-available forms, which is highly beneficial for agricultural applications as a biofertilizer.

Antagonistic Activity of PGPR Isolates Against *Colletotrichum scovillei*

The observations revealed that all tested PGPR isolates possessed the capability to inhibit the growth of *C. scovillei* compared to the control. During the initial phase (3 dai), isolate A 2 exhibited the highest inhibition at 46.3%. By (7 dai), effectiveness fluctuated; while isolate A 2 maintained the highest inhibition (49.46%), isolates B 5.2 and B 5.7 experienced a drastic decline to below 1%. This suggests that the pathogen's growth rate initially outpaced the diffusion rate of the isolates' antimicrobial compounds. However, by the final observation (14 dai), a significant increase in inhibition was observed across nearly all isolates. The three top performing isolates at (14 dai) were A 11 (62.93%), B 5.13 (62.2%), and A 13 (61.83%). This terminal increase in inhibition indicates that the bacteria require an incubation period to reach the stationary phase and produce secondary

metabolites and hydrolytic enzymes such as chitinase and protease at sufficient concentrations to permanently suppress pathogen expansion.

Table 5. Inhibitory Activity of PGPR Isolates Against the Growth of *C. scovillei*

Treatment	3 (dai)	7 (dai)	14 (dai)
Control	0	0 IJ	0 i
A 1	22.3 cdefg	14.117 efghij	32.63 efgh
A 2	46.3 a	49.46 A	56.93 abc
A 3	36.13 ab	33.53 b	47.17 cd
A 4	34.57 bc	27.93 bcd	36.57 efg
A 5	25.86 bcdf	8.67 fghij	50.13 bcd
A 6	25.97 bcdef	7.03 fghij	32.13 efgh
A 9	29.2 bcde	7.97 fghij	28.93 gh
A 10	20.73 cdefgh	1.77 hij	18.73 h
A 11	37.8 ab	34.47 b	62.93 a
A 13	30.9 bcd	30.97 bc	61.83 ab
A 14	32. 7 bc	16.73 defghi	33 efgh
B 2.1	10.26 fghij	4.37 ghij	28.63 gh
B 2.2	10.33 fghij	5.27 ghij	32 efgh
B 3.3.1	12 fghij	7.07 fghij	28.27 gh
B 4.2	8.6 ghij	5.23 ghij	32 efgh
B 4.4	10.33 fghij	7.03 fghij	32 efgh
B 5.2	5.2 hij	0.1 ij	32.36 efgh
B 5.3	17.2 defghi	20.37 cdefg	52.8 abcd
B 5.5	3.43 ij	22.1 bcdef	50.9 abcd
B 5.6	7 ghij	1,6 hij	44.97 cde
B 5.7	1.67 ij	0.9 j	27.1 gh
B 5.9	10.26 fghij	3.5 hij	30.83 efgh
B 5.11	20.77 cdefgh	7.03 fghij	29.77gh
B 5.13	20.7 cdefgh	26.73 bcde	62.2 ab
B 5.14	8.6 ghij	7.03 fghij	31.27 efgh
C 1.3	15.73 efghij	5.03 ghij	43.77 def
C 1.4.1	15.53 efghij	7.03 fghij	29.03 gh
C 2.4.1	17.2 defghi	7.93 fghij	31.23 efgh
C 2.7	13.67 fghij	8.83 fghij	29.77 fgh
C 3.1	12 fghij	6.17 fghij	32 efgh
C 3.2	12 fghij	7.03 fghij	30.5 fgh
C 4.2	15.53 efghij	7.03 fghij	31.63 efgh
C 4.5	10.36 fghij	5.23 ghij	27.13 gh
C 5.1	12 fghij	14.97 defghi	25.6 gh
C 5.2	15.53 efghij	18.52 cdefgh	51.67 abcd
C 5.3.1	12 fghij	9.66 fghij	32 efgh
C 5.3.2	17.2 defghi	7.93 fghij	32 efgh

The significant increase in inhibition by (14 dai) indicates that PGPR biological agents require an incubation period to reach the stationary phase and produce secondary metabolites or antibiotics at

concentrations sufficient to optimally suppress *C. scovillei* growth. Isolates from Cikahuripan Village tended to provide a more stable inhibitory response from the initial observation compared to those from Leuwimalang and Ciherang villages. This suggests superior adaptability and colonization speed within the Cikahuripan group regarding niche competition for space and nutrients. The decline in inhibitory activity observed on day 7 for certain isolates is likely related to shifts in bacterial growth phases or environmental factors temporarily affecting the production of antifungal compounds. However, the surge in inhibition by (14 dai), particularly for isolates A 11 and B 5.13 which exceeded 60%, demonstrates their robust potential as biocontrol agents. According to [25] long term antagonistic effectiveness is often driven by the continuous accumulation of hydrolytic enzymes and lipopeptide compounds that increase throughout the incubation period

4. Conclusion

This study successfully isolated 80 indigenous Plant Growth-Promoting Rhizobacteria (PGPR) from the chili rhizosphere in West Java using an innovative selection method with media supplemented with 2% biopesticide nanoemulsion. The selection resulted in a dominance of Gram-positive bacteria (62.5%), indicating high environmental resilience. Based on safety and functional evaluations, 43 non-pathogenic isolates (HR negative) were selected, exhibiting significant multifunctional potential. These include 7 antagonistic isolates, 2 phosphate-solubilizing isolates (A 11 and C 5.2), and broad enzymatic activities including chitinase (7 isolates), amylase (9 isolates), and protease (14 isolates). Specifically, isolates C 5.2 and A 11 were identified as elite candidates due to their comprehensive enzymatic profiles and growth-promotion capabilities. This research concludes that PGPR selected through nanoemulsion-supplemented media hold great potential as multifunctional biological agents compatible with modern agricultural technologies to enhance sustainable chili productivity.

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