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Potential of Some *Bacillus* Rhizobacteria as Biofertilizer and Biocontrol

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Abstract

Biofertilizers and biocontrol agents can reduce the use of chemical inputs in agricultural production and are classified as environmentally friendly agents. *Bacillus* rhizobacteria were a group of microorganisms that could serve as biofertilizers as well as biocontrol agents. This study has explored the potential of some *Bacillus* strains as biofertilizers and biocontrol agents. A total of 17 *Bacillus* isolates were collected from various rhizospheres of plants and different soil types in South Sulawesi, Indonesia. These isolates were identified at the strain level through 16S rRNA gene sequencing and evaluated for their biofertilizer ability to N fixation, P and K solubilization, thermotolerance, GA3 and IAA exudation and biocontrol potential (*i.e*., proteolytic, chitinolytic and cellulolytic). The ability of each *Bacillus* isolate was different, even within the same strain. Isolates LSi-3 (*B. albus* strain MCCC 1A02146), JSi-4 (*B. cereus* IAM 12605), PGa-1.2 (*B. albus* strain MCCC 1A02146), KW0-2.2 (*B. cereus* strain SJ37), KWo-2.1 (*B. proteolyticus* strain MCCC 1A00365), BMBe-3 (*B. albus* strain VIT-RPJ) and BGa-2.1 (*B. proteolyticus* strain MCCC 1A00365) were highly promising for use as biofertilizers and biocontrol agents, either individually or in combination. Isolates BGa-2.2 (*B. cereus* strain IAM 12605) and JBe-4 (*B. tropicus* strain AOA-CPS1) had the potential to be used with other rhizobacteria, which could enhance the exudation of IAA by biofertilizers.

Keywords: Hydrolytic enzyme; N, P, K solubilisation; Phytohormone; Rhizobacteria

Introduction

An increase in food production is necessary to meet the needs of the growing global population. However, most of the increase in food production is achieved through intensive farming systems that rely heavily on synthetic chemicals, such as fertilizers and pesticides, to boost plant growth and suppress pests and diseases. The long-term use of chemical fertilizers for agricultural production negatively impacts the environment. Excessive use of inorganic fertilizers is significantly correlated with a reduction in biodiversity (Mozumder and Berrens 2006). Thus, there is a need to develop alternative fertilizers that are inexpensive, effective, sustainable, and environmentally friendly (Javaid and Bajwa 2011; Pirttilä *et al.* 2021).

The use of chemical pesticides poses significant risks to the environment and non-target organisms, *i.e*., beneficial soil microorganisms, insects, plants, fish and birds, as well as causing pathogen resistance (Sharma and Singhvi 2017; Pirttilä *et al.* 2021). Furthermore, intensive use of chemical pesticides hurts soil environments, as pesticide residues persist in the soil for a considerable amount of time. Most pesticides negatively affect microorganisms' biological function, diversity, composition and biochemical processes (Meena *et al.* 2020).

Efforts to reduce chemical usage and promote environmentally friendly agriculture include the utilization of rhizobacteria as biofertilizers and biocontrol (Shoaib *et al.* 2020; Javed *et al.* 2021). Rhizobacteria are bacteria that colonize plant roots and can fix nitrogen, solubilize nutrients, produce plant growth hormones, produce fungal growth inhibitors, and control plant pathogens without disrupting plants and other ecosystem components (Li *et al.* 2017; Mendes *et al.* 2020; Poria *et al.* 2021).

Bacillus is a commonly used rhizobacterium for biofertilization and biocontrol) (Sharf *et al.* 2021). The genus *Bacillus* has multifunctionality, acting as a biofertilizer that promotes plant growth by increasing nutrient availability and phytohormone production. Additionally, *Bacillus* can be a biocontrol with antagonistic activity by releasing extracellular metabolites such as antibiotics, cell wall hydrolases and siderophores (Ma *et al.* 2018; Miljakovi'c *et al.* 2020). Various plants have shown positive effects of the application of *Bacillus* spp. on growth and yield, including tomato, potato, cucumber, maize, beans, soybean, sunflower, wheat, pepper, rice and many others (Akinrinlola *et al.* 2018; Husna and Pratiwi 2020; Khan *et al.* 2022). Each strain of rhizobacteria has a

different biofertilizer and biocontrol potential. This is due to differences in their ability to increase nutrient availability and produce hormones and enzymes that inhibit plant pathogens. Therefore, it is necessary to evaluate the potential of each strain for use as a biofertilizer and biocontrol. This study aimed to determine the potential of some *Bacillus* rhizobacteria as biofertilizers and biocontrol.

Materials and Methods

Isolation and identification of *Bacillus* from the plant rhizosphere

Rhizobacteria isolates were obtained from various plant rhizospheres, including maize, taro, cocoa, soybean, pine, bamboo, carrot, pepper and grass, in several locations in the province of South Sulawesi, Indonesia (Table 1). The bacterial isolation was performed by serial dilution method up to 10^8. One gram of soil was dissolved in 10 mL of distilled water and shaken for 30 min. Then, 1 mL of the soil suspension was added to a test tube containing 9 mL of distilled water. Subsequently, 0.1 mL of the final suspension was cultured on Nutrient Agar (NA) medium in a Petri dish and incubated at 28°C for 24 h.

The identification of selected *Bacillus* rhizobacteria isolates at the strain level was conducted through molecular methods, specifically sequencing of the 16S rRNA gene. Bacterial DNA was isolated and purified using the Quik-DNA TM fungal/bacterial Miniprep Kit (D6005). The isolated DNA was then amplified using a pair of primers: 27F (5' AGAGTTTGATCCTGGCTAG 3') and 1492R (5' TACGGYTACCTTGACGACTT 3'). The Polymerase Chain Reaction (PCR) product was electrophoresed using MyTag HS Red Mix (Bioline) and visualized under a UV transilluminator. The DNA amplification of the selected isolate resulted in a 1400 bp product. The sequencing results were used to search for homologous 16S rRNA sequence matches in the DNA database (GenBank) using the Basic Local Alignmen Search Tool (BLAST) program by the National Centre for Biotechnology Information (NCBI).

Determine nitrogen fixation, phosphate and potassium solubilization

The ability of the bacterial isolates to fix nitrogen was tested using Ashby's Mannitol agar medium (composed of 20 g mannitol, 0.2 g K_2HPO_4, 0.1 g NaCl, 0.1 g K_2SO_4, 0.2 g $MgSO_4.7H_2O$, 5 g $CaCO_3$, 20 g agarose, and distilled water to make 1 L). Each isolate was streaked onto the surface of the Ashby's Mannitol agar medium and incubated at room temperature for seven days. The presence of a clear zone around the bacterial colonies indicates the ability to fix nitrogen (Nader *et al*. 2019).

The ability of bacterial isolates to solubilize phosphate was tested using Pikovskaya's agar medium with the following composition: 10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g yeast extract, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.2 g NaCl, 0.1 g $MgSO_4.7H_2O$, a small amount of $MnSO_4$ and $FeSO_4$, 20 g agarose and 1 L distilled water. Each isolate was streaked onto the surface of Pikovskaya's agar medium and incubated for seven days at room temperature. Clear zones around the bacteria indicated their ability to solubilize phosphate (Nader *et al*. 2019).

The potassium solubilisation ability of bacterial isolates was tested using Alexandrov agar medium (containing 5 g glucose, 0.5 g $MgSO_4.7H_2O$, 0.006 g $FeCl_3$, 0.1 g $CaCO_3$, 2 g Ca_3PO_4, 3 g K_2HPO_4, 20 g agar and 1000 mL distilled water). Each *Bacillus* isolate was streaked onto the surface of Alexandrov agar medium and incubated at room temperature for seven days. Clear zones around bacterial colonies indicated the ability to solubilize potassium (Zhang and Konga 2014).

Phytohormones (indole acetic acid and gibberellin acid) production

The ability of bacteria to produce IAA was tested by growing them in Nutrient Broth (NB) supplemented with L-tryptophan at 0.1 g L^{-1}, at a temperature of 28°C in the dark for seven days. The supernatant was then centrifuged and 1 mL was transferred to a reaction tube containing 1 mL of Salkowski's reagent (composed of 150 mL H_2SO_4, 250 mL sterile distilled water (SDW) and 7.5 mL $FeCl_3.6H_2O$ 0.5 M) and stored at room temperature in the dark for 24 h. A pink colour change in the culture indicates the presence of IAA production. The concentration of IAA was measured using a spectrophotometer at a wavelength of 535 nm with an IAA standard (Gusmiaty *et al*. 2019).

To determine the ability of bacteria to produce gibberellic acid (GA_3), they were grown on Nutrient Broth (NB) medium. A 1 mL bacteria isolate was inoculated into the NB medium and incubated at room temperature for 7 days, followed by centrifugation for 15 min. Next, 15 mL of the supernatant was mixed with 2 mL of zinc acetate and then 2 mL of potassium ferrocyanide was added after 2 min, followed by centrifugation for 10 min. Then, 5 mL of the mixture was added to a test tube containing 30% hydro colloidal acid and incubated for 75 min at 28°C. GA3 was measured using a spectrophotometer at a wavelength of 254 nm with a GA3 standard (Gusmiaty *et al*. 2019).

Thermotolerant bacteria

The purpose of the thermotolerance test was to obtain bacteria that were resistant to high temperatures. Heat-resistant bacterial isolates were tested by incubating the bacterial isolate suspension at 50°C. One hundred microliters of the isolate were added to 10 mL of NB medium and then incubated for 72 h. After incubation, the bacterial isolate suspension is regrown on Nutrient Agar and its growth is observed after 24 h. The growth of the isolate indicates that it is resistant to the temperature tested, and the

Table 1: Isolate code, plant rhizosphere and soil properties of rhizobacteria collection in South Sulawesi, Indonesia

No.	Isolate code	Plant rhizosphere	Coordinates and latitude	Soil properties
1	JMs-3	Maize	4º54'33.8''S 119º51'49.7''E 306 masl. (metre above the sea level)	Calcareous soil, pH 8.2
2	TMs-4	Taro	5º07'27.0''S 119º36'48.0''E 91 masl.	Acidic soil, pH 5.2
3	CBe-2.2	Cacao	4º31'25.7''S 120º07'58.5''E 251 masl.	Calcareous soil, pH 7,6
4	BMBe-3	Shallot	4º35'42.3''S 120º15'46.6''E 100 masl.	Calcareous soil, pH 8
5	JBe-4	Maize	4º14'34.1''S 120º13'52.4''E 102 masl.	Calcareous soil, pH 7.5
6	KWo-2.1	Soybean	4°03'00.4"S 120°01'11.4"E 30 masl.	Normal soil pH 7
7	KWo-2.2	Soybean	4°03'00.4"S 120°01'11.4"E 30 masl.	Normal soil, pH 7
8	PGa-1.2	Pinus	5º14'22.1''S 119º38'20.3''E 139 masl.	Normal soil, pH 6.7
9	PGa-1.3	Pinus	5º14'22.1''S 119º38'20.3''E 139 masl.	Normal soil, pH 6.7
10	BGa-2.1	Bamboo	5º16'47.8''S 119º45'49.7''E 407masl.	Normal soil, pH 7
11	BGa-2.2	Bamboo	5º16'47.8''S 119º45'49.7''E 407 masl.	Normal soil, pH 7
12	WGa-3	Carrot	5º15'08.5''S 119º55'17.7''E 1602 masl.	Normal soil, pH 6.7
13	WGa-3.1	Carrot	5º15'08.5''S 119º55'17.7''E 1602 masl.	Normal soil, pH 6.7
14	JSi-1	Maize	5º14'18.1''S 119º59'51.1''E 1021 masl.	Normal soil, pH 6.9
15	LSi-3	Pepper	5º13'19.1''S 120º08'16.5''E 220 masl.	Acidic soil, pH 5.8
16	JSi-4	Maize	5º13'59.3''S 120º08'27.3''E 356 masl.	Normal soil, pH 6.9
17	RBg-1	types of grass	5º35'12.9''S 120º05'08.8''E 5 masl.	Saline soil, pH 8.5

population of living bacterial colony isolates is calculated (Sari *et al*. 2012). The number of colonies that grow is then converted into cfu mL^{-1} units using the formula:

$$\text{Bacterial population} = \frac{\mathbf{X}}{\mathbf{p} \times \mathbf{v}} \qquad (1)$$

Where:
X = The number of colonies that grew on the petri dish with a dilution factor of (cfu).
p = dilution factor of
v = The suspension volume spread on the petri dish (mm).

Determine of chitinase, cellulase and protease of isolates activity

The chitinolytic testing that is commercial chitin colloid and crab shell chitin was prepared by adding 20 g (of commercial and crab shell chitin) into 300 mL of concentrated HCl and homogenizing the mixture. The solution was then incubated in a refrigerated cabinet for 24 h and 200 mL of cold distilled water was added and left to stand overnight at 4°C. The solution was then filtered using glass wool, and the filtrate was neutralized with 12 N

NaOH to pH 7. The solution was then centrifuged at 4,000 rpm for 10 min and the resulting precipitate was washed with sterile distilled water and centrifuged again at 4,000 rpm for 10 min. The bacterial isolates were streaked onto chitin agar medium (0.05% $MgSO_4.7H_2O$, 0.07% K_2HPO_4, 0.1% yeast extract, 0.5% crab shell chitin colloid and 1.5% agar) and incubated at room temperature for 48–120 h. The diameter of the halo zone was then observed (Whipps 2001).

The protease production was determined by the procedures Brown and Foster (1970), *i.e*., skim Milk Agar medium is prepared by dissolving 10 g of skim milk in 100 mL of distilled water, heating the solution on a hot plate until dissolved and sterilizing it at 110°C for 15 min. Furthermore, 18 g of nutrient agar (NA) is dissolved in 900 mL of distilled water, boiled on a hot plate, homogenized using a magnetic stirrer, and sterilized at 121°C for 15 min. The NA medium is mixed homogeneously with the skim milk medium while hot. The resulting medium is then poured into 9 cm diameter Petri dishes. One colony of bacteria is inoculated onto the medium and incubated at room temperature for 24 h. The presence of a halo zone surrounding the bacterial colony indicates proteolytic activity.

Cellulolytic test using the procedure of Emtiazi *et al*. (2007), *i.e*., rhizobacteria isolate was cultivated on carboxymethyl cellulose (CMC) medium, which was composed of $MgSO_4.7H_2O$ (0.05 g 100 mL^{-1}), $Na_2HPO_4.2H_2O$ (0.5 g 100 mL^{-1}), NaCl (0.23 g 100 mL^{-1}), yeast extract (0.2 g 100 mL^{-1}), CMC (1 g100 mL^{-1} and agar (2.5 g 100 mL^{-1}). A single colony was inoculated and incubated for 24 h at 35°C. The colony was then stained with 0.1% Congo red, incubated for 30 min, then rinsed with a 1% NaCl solution. A halo zone around the colony was observed and measured for its diameter.

Data analysis

The ability to fix N and solubilize P and K were classified into four categories, namely: (-) negative indicating the absence of halo zones, (+) halo zone < 2 cm (low), (++) halo zone 2–3 cm (moderate) and (+++) halo zone > 3 cm (high).

Comparison of the data on IAA, GA, thermotolerance, chitinolytic and proteolytic activity were analyzed using the following equations:

$$Xi = \overline{X} \pm Sd \qquad (2)$$

Where:
Xi = comparison data
$\overline{X}$ = Average of all data
Sd= standard deviation from all data

Criteria: if X = 0 (no value), X > X i ($\overline{X}$ + Sd) is high, X = X i = value between ($\overline{X}$ - Sd) and ($\overline{X}$ + Sd) is medium, and X < Xi ($\overline{X}$ - Sd) is low

Determining rhizobacteria that are superior to other bacteria is done by scoring each bacterium with a value of 0 – 3, namely 0 = no value, 1 = low, 2 = medium and 3 = high. The highest total value is considered superior bacteria.

Results

Morphology of bacterial isolates

The isolated rhizobacteria were identified as *Bacillus* spp. based on their Gram-positive reaction and morphology. The colony morphology exhibited variations, ranging from irregularly circular, irregularly circular with rough margins, to undulated circular with elevated and flat edges. The colonies were white and pale yellow (Table 2).

Strain identification using 16s rRNA gene partial sequences

After the identification of isolates using 16S rRNA gene sequence homology analysis with GenBank database, 17 isolates were identified as *Bacillus*, with the following strains obtained: 1) *B. proteolyticus* strain MCCC 1A00365, which were isolated from maize, soybean, bamboo and carrot plants (isolates JMs-3, KW_0-2.1, BGa-2.1 and WGa-3.1, respectively), 2) *B. cereus* strain XS.7-1 (TMs-4), which was isolated from taro plants, 3) *B. cereus* strain IAM 12605, which were isolated from bamboo and maize plants (isolates BGa-2.2 and JSi-4, respectively), 4) *B. cereus* SJ37 (KWo-2.2), which was isolated from soybean plants, 5) *B. cereus* strain B.30 (PGa.1-3), which was isolated from pine plants, 6) B. cereus BXC15 (Isolate JSi-1), which was isolated from maize plants, 7) *B. parantracis* MN1F (CBe-2.2), which was isolated from cacao plants, 8) *B. albus* strain VIT-RPJ (BMBe-3), which was isolated from shallot plants, 9) *B. albus* strain MCCC 1A02146, which were isolated from pine, chili, and rice plants (isolates PGa-1.2, LSi-3 and RBg-1, respectively), 10) *B. tropicus* strain AOA-CPS1 (JBe-4), which were isolated from maize, pepper and grass plants, 11) *B. paramycoides* strain MCC1A04098 (WGa-3), which was isolated from carrot plants. The level of similarity between the isolates was 99.65 to 100% (Table 3).

Nitrogen fixation, phosphate and potassium solubility

The results of this study indicated that of the 17 isolates of *Bacillus* rhizobacteria (Table 4) which showed the formation of a halo zone on Ashby's Mannitol Agar media, two isolates were revealed that could fix N, namely Kwo-21 (*B. proteolyticus* strain MCCC 1A00365) and PGa-1.2 (*B. albus* strain MCCC 1A02146). P solubility using Pikovskaya media were isolates KWo-2.1 and BGa-2.1, both of which were *B. proteolyticus* strain MCCC 1A00365, as well as isolates PGa-1.2 (*B. albus* strain MCCC 1A02146) and PGa-1.3 (*B. cereus* strain B.30), while the solubility of K using Alexandrov media showed that all tested *Bacillus* isolates could dissolve K, except for isolates Kwo-2.1 and BGa-2.2. The ability to dissolve K. BGa-2.1 (*B. proteolyticus* strain

Table 2: Morphological characterization of bacterial isolates

No.	Isolate code	Colony form	Edge of the colony	Colony elevation	Colony colour	Gram reaction
1	JMs-3	Round	Choppy	Appear	White	(+)
2	TMs-4	Round	Complete	Appear	Yellowish white	(+)
3	CBe-2.2	Round	Choppy	Appear	White	(+)
4	BMBe-3	Round	Choppy	Appear	White slightly translucent	(+)
5	JBe-4	Round	Complete	Appear	White	(+)
6	KWo-2.1	Round	Choppy	Appear	White	(+)
7	KWo-2.2	Round	Choppy	Appear	Clear white	(+)
8	PGa-1.2	Round	Choppy	Appear	White	(+)
9	PGa-1.3	Round	Choppy	Appear	White	(+)
10	BGa-2.1	Round	Choppy	Flat	White	(+)
11	BGa-2.2	Round	Choppy	Appear	White	(+)
12	WGa-3	Round	Choppy	Appear	Beige	(+)
13	WGa-3.1	Round	Choppy	Appear	White	(+)
14	JSi-1	Round	Complete	Appear	White	(+)
15	LSi-3	Round	Jagged	Flat	White	(+)
16	JSi-4	Irregular	Choppy	Flat	White	(+)
17	RBg-1	Round	Jagged	Appear	White	(+)

Table 3: DNA sequencing result of several *Bacillus* sp.

No	Isolate	Bacillus strain	Similarity (%)	Assessment No.
1	JMs-3	*Bacillus proteolyticus* Strain MCCC 1A00365	99.93	NR157735.1
2	TMs-4	*Bacillus cereus* strain XS.7-1	100	MT1000007.1
3	CBe-2.2	*Bacillus paranthaccis strain* MN1F	99.93	CP046887.1
4	BMBe-3	*Bacillus albus* strain VIT-RPJ	99.86	KJ437475
5	JBe-4	*Bacillus tropicus strain* AOA-CPS1	100	CP0491
6	KWo-2.1	*Bacillus proteolyticus* Strain MCCC 1A00365	99.93	NR157735
7	KWo-2.2	*Bacillus cereus* strain SJ37	100	NT103054
8	PGa-1.2	*Bacillus albus* strain MCCC 1A02146	99.33	NR157729
9	PGa-1.3	*Bacillus cereus strain B.30*	99.74	LN890206.1
10	BGa-2.1	*Bacillus proteolyticus* strain MCCC 1A00365	99.79	NR157735
11	BGa-2.2	*Bacillus cereus* strain IAM 12605	99.86	NR115526
12	WGa-3	*Bacillus paramycoides* strain MCCC 1A04098	99.81	NR157734.1
13	WGa-3.1	*Bacillus proteolyticus* strain MCCC 1A00365	99.86	NR157735
14	JSi-1	*Bacillus cereus* strain BXC15	100	MN227492.1
15	LSi-3	*Bacillus albus strain* MCCC 1A02146	100	NR157729
16	JSi-4	*Bacillus cereus* strain IAM 12605	99.65	NR115526
17	RBg-1	*Bacillus albus* strain 1A02146	99.74	NR157729

Table 4: Bacterial isolates screened for N-fixation, P-solubility, and K-solubility

No	Isolate code	N- fixation	P-solubility	K- solubility
1	JMs-3	-	-	++
2	TMs-4	-	-	+
3	CBe-2.2	-	-	+
4	BMBe-3	-	-	+
5	JBe-4	-	-	++
6	KWo-2.1	++	+++	-
7	KWo-2.2	-	-	+
8	PGa-1.2	+	++	-
9	PGa-1.3	-	+	+
10	BGa-2.1	-	++	+++
11	BGa-2.2	-	-	++
12	WGa-3	-	-	+
13	WGa-3.1	-	-	+
14	JSi-1	-	-	++
15	LSi-3	-	-	++
16	JSi-4	-	-	+++
17	RBg-1	-	-	+++

Description: (-) no halo zone, (+) halo zone <2 cm (low), (++) halo zone 2-3 cm (medium), and (+++) halo zone >3 cm (high)

MCCC 1A00365), JSi-4 (*B. cereus* strain IAM 12605) and RBg-1 (*B. albus* strain 1A02146) has a relatively higher ability to solubilize K than other isolates.

Exudation of phytohormones

The results of this study indicate that all tested *Bacillus*

isolates can produce indole acetic acid (IAA) ranging between 13.29–101.28 mg.kg^{-1} (Table 5). Isolates JBe-4 (*B. tropicus* strain AOA-CPS1) and BGa-2.2 (B. cereus strain IAM 12605) produce higher amounts of IAA than other isolates, with 101.28 and 90.17 mg.kg^{-1}, respectively. JBe-4 and BGa-2.2 also produce GA3 with 19.12 and 12.94 mg.kg^{-1}, respectively.

All tested isolates could produce GA3 between 12.94 to 28.08 mg.kg^{-1}. Isolate LSi-3 *(B. albus* strain MCC 1A04098) and JSi-4 (*B. cereus* strain IAM 12605) produced higher levels of GA3 than other isolates, at 25.79 mg.kg^{-1} and 28.06 mg.kg^{-1}, respectively. These two isolates also produced IAA at 64.14 mg.kg^{-1} and 62.25 mg.kg^{-1}, respectively (Table 5).

Thermotolerant bacteria

Testing thermotolerance on 17 bacterial isolates showed a reduction in population size with increasing temperature. However, all isolates tested were able to grow up to 50°C, with populations ranging from 8.8×10^6 to 266.4×10^6 cfu mL^{-1}. Isolates PGa-1.2 (*B. proteolyticus* strain MCCC 1A02146), BGa-2.2 (*B. cereus* strain IAM 12605), RBg-1 (*B. albus* strain MCCC 1A02146), BGa-2.1 (*B. proteolyticus* strain MCCC 1A00365) and WGa.-3.1 (*B. proteolyticus* strain MCCC 1A00365) were classified as thermotolerant at a temperature of 50°C, with their populations showing a significant increase, surpassing 194.69×10^6 cfu mL^{-1} (Table 5).

Chitinolytic, proteolytic, and cellulolytic activity as biocontrol

In this study, several rhizobacteria bacteria could produce hydrolytic enzymes such as chitinase, proteinase and cellulose, as indicated by the formation of clear zones around bacterial colonies. The chitinolytic index ranged from 0.13 to 0.73, the proteolytic index ranged from 0.13 to 1.71 and the cellulolytic index ranged from 0.04 to 1.65 (Table 6). The isolates KWo-2.2 (B. *cereus* strain SJ37) and WGa-3 (*B. paramycoides* strain MCCC1A04098) exhibited high chitinolytic activity, with respective chitinolytic indices of 0.46 and 0.73. strain .7-1), Wo-2.2, and PGa-1.2 exhibited high proteolytic indices, ranging from 0.92 to 1.71, compared to the other isolates. Meanwhile, isolates WGa-3 and BMBe-3 (*B. albus* strain VIT-RPJ) exhibited high cellulolytic indices, with respective indices of 0.63 and 1.63 (Table 6).

Discussion

Bacillus rhizobacteria could live around the roots of plants that grow in acid-alkaline soils. Notably, *Bacillus* spp. has been reported to have a broad pH range for optimal growth, from pH 5.0 to 9.0 (Parab *et al.* 2020). The differences in the plant rhizospheres and the growing environment caused morphology, population, rhizobacteria strains, and physiology variations. The diversity of soil conditions and plant rhizospheres is responsible for the diverse rhizobacterial isolates that can be obtained (Hasra and Pratiwi 2013; Liu *et al.* 2016; Oo *et al.* 2020). The level of similarity between the isolates was 99.65 to 100% (Table 3). This level of similarity is high because, using the 16S rRNA marker, the identity is considered similar at the species level when the "percentage identity" value is > 97.5% and at the genus level when the "percentage identity" value is > 95% (Stackebrandt and Goebel 1994).

Macronutrients nitrogen (N), phosphorus (P) and potassium (K) are essential for plant growth and productivity, and their deficiency can significantly affect crop yields. Even though chemical fertilizers are added to the soil, plants cannot utilize all the added fertilizer. Therefore, using highly efficient rhizobacteria will practically increase the nutrient content in the plant rhizosphere. The enhancement of nutrient availability and plant growth by inoculation with N-fixation, P, or K-solubilizing rhizobacteria has been extensively studied and documented by many researchers (Zhang *et al.* 2017; Gupta *et al.* 2021; Sembiring *et al.* 2021). This research showed that some *Bacillus* have a dual function in the availability of plant nutrients, as indicated by the presence of N fixation and the solubilization of P and K. Isolates KWo-2.1 (*B. proteolyticus* strain MCCC 1A00365) and PGa-1.2 (*B. albus* strain MCCC 1A02146) are good at fixing N and solubilizing P but are unable to solubilize K. These isolates (KWo-2.1 and PGa-1.2) were grown well on Ashby's Mannitol and Pikovskaya's agar medium. Ashby's Mannitol media did not contain nitrogen, so the growing bacteria showed that these bacteria can fix nitrogen in the atmosphere. Whereas, Pikovskaya's agar medium contains $Ca_3(PO_4)_2$, therefore the rhizobacteria identified as P solubilizers in this medium, as indicated by the formation of halozones, are also able to convert the form of phosphate that is not available to plants [$Ca_3(PO_4)_2$] into the available form (HPO_4^{2-} or $H_2PO_4^-$). In similar research, *Bacillus* spp. could fix N and solubilize P (Sahin *et al.* 2004; Husna and Pratiwi 2020). Isolates PGa-1.3 and BGa-2.1 were unable to fix N, but they could solubilize P and K. Isolates JMS-3, TMs-4, CBe-2.2, BMBe-3, JBe-4, KWo-2.2, BGa-2.2, WGa-3, WGa-3.1, JSi-1, LSi-3, JSi-4 and RBg-1 isolates could only solubilize K, whereas the Ga-2 isolate could only solubilize P.

IAA and GA3 are both essential phytohormones that may function as the main regulators of plant growth and reproduction (Asif *et al.* 2022). In this research, all *Bacillus* isolates were tested to produce Indole Acetic Acid (IAA) and Gibrellin Acid (GA_3). Similarly, the research findings of Gusmiaty *et al.* (2019) and Mendes *et al.* (2020) indicate that more than 80% of bacteria isolated from the rhizosphere can produce IAA and gibberellin. The production of IAA by bacteria varies depending on species and strain. Differences in morphological characteristics of rhizobacterial colonies support the variation of bacterial strains in producing IAA

Table 5: Bacterial isolates screened for exudation phytohormone and thermotolerant

No.	Isolate Code	Phytohormone (mg.kg^{-1})		Population of thermotolerant bacteria (cfu mL^{-1})
		IAA	GA3	
1	JMs-3	83,46	19,17	83,2 x 10^6
2	TMs-4	44,44	16,89	96,6 x 10^6
3	Be-2	53,50	16,73	9,6 x 10^6
4	BMBe-3	79,05	19,89	25,9 x 10^6
5	JBe-4	101,28	19,12	17,4 x 10^6
6	KWo-2.1	59,31	14,79	29 x 10^6
7	KWo-2.2	66,87	17,58	140 x 10^6
8	PGa-1.2	55,20	17,01	266,4 x 10^6
9	PGa-1.3	13,29	13,53	159,2 x 10^6
10	BGa-2.1	71,66	19,19	227,6 x 10^6
11	BGa-2.2	90,17	12,94	244,7 x 10^6
12	WGa-3	65,49	14,79	11,7 x 10^6
13	WGa-3.1	78,98	14,13	203,2 x 10^6
14	JSi-1	76,06	16,08	8,8 x 10^6
15	LSi-3	64,14	25,79	22,3 x 10^6
16	JSi-4	62,25	28,06	13,7 x 10^6
17	RBg-1	54,96	19,74	241,8 x 10^6
	Average	65.89	17,97	101,95 x 10^6
	Std	19.26	3,92	92,74 x 10^6
	Height	> 85.15	> 21,894	> 194,69 x10^6
	Medium	46.67-85.15	13.59-21.89	(9,2 - 194,69) x 10^6
	Low	< 46.67	< 13,59	< 9,2 x 10^6

(Kumar *et al.* 2014; Lestari *et al.* 2017). IAA produced by *Bacillus* isolates significantly improves the vigor of rice and corn seeds when inoculated with the bacteria (Pakhtunkhwa *et al.* 2017; Chandra *et al.* 2018). The use of *Bacillus* rhizobacteria, which can exudate the hormone gibberellin, can improve plant growth (Kang *et al.* 2014; Desai 2017; Kang *et al.* 2019). The production of IAA and GA3 by rhizobacteria suggests using these bacteria as a potential source of biofertilizers to enhance plant growth and productivity, especially under adverse environmental conditions. Thus, identifying rhizobacteria with IAA and GA3-producing abilities and their subsequent use as biofertilizers could have important implications for sustainable agriculture.

The use of rhizobacteria as a biocidal agent has gained increasing attention due to its potential as an eco-friendly alternative to pesticide use, which is non-resistant to target organisms and has a stimulating effect on plant growth. One of the mechanisms by which bacterial strains become biocidal agents is by degrading the cell wall of pathogenic microorganisms through the production of hydrolytic enzymes (Roca-couso *et al.* 2021; Khan *et al.* 2022). Testing the chitinolytic, proteolytic and cellulolytic activities of bacteria aims to determine the presence of chitinase, protease, and cellulase enzymes produced by the bacteria. These enzymes play an important role in controlling plant pests and diseases by degrading the cell walls of phytopathogens (Akinrinlola *et al.* 2018; Oo *et al.* 2020). In this study, several rhizobacteria bacteria could produce hydrolytic enzymes such as chitinase, proteinase, and cellulose, as indicated by the formation of clear zones around bacterial colonies. *Bacillus* spp. can produce hydrolytic enzymes such as chitinase, gluconase, cellulase, lipase, and protease, which can hydrolyze the main components of fungal cell walls and suppress the development of plant pathogens (Bonaterra *et al.* 2022). Enzyme-producing bacteria capable of hydrolyzing protein, cellulose and chitin on test media can inhibit the growth of *Fusarium* spp. (Meliah *et al.* 2020). The antagonistic properties of hydrolytic enzymes against various phytopathogens play a significant role in biocidal activity (Jadhav *et al.* 2017).

Table 6: Mean values of chitinolytic, proteolytic and cellulolytic activity indices of bacterial isolates after 24-hour incubation

No.	Isolate Code	Activity index		
		Chitinolytic	Proteolytic	Cellulolytic
1	JMs-3	0.23	0.13	0.44
2	TMs-4	0	0.92	0.08
3	Be-2	0	0	0.04
4	BMBe-3	0.27	0.26	1.65
5	JBe-4	0.31	0.48	0
6	KWo-2.1	0.19	0.15	0
7	KWo-2.2	0.46	1.03	0,4
8	PGa-1.2	0.17	1.71	0.11
9	PGa-1.3	0.16	0.68	0
10	BGa-2.1	0.17	0.30	0
11	BGa-2.2	0	0.22	0
12	WGa-3	0.73	0.58	0.63
13	WGa-3.1	0	0.44	0
14	JSi-1	0.13	0.45	0
15	LSi-3	0.45	0.27	0.48
16	JSi-4	0.31	0.53	0.47
17	RBg-1	0.37	0	0
	Average	0,30	0,54	0,55
	Std	0,16	0,40	0,49
	Height	> 0,46	> 0,94	> 1.04
	Medium	0.14 - 0.46	0.14 - 0.94	0.06 - 1.04
	Low	< 0,14	< 0, 14	< 0.06

The results of this study demonstrate that *Bacillus* spp. has significant potential as an alternative to inorganic or chemical fertilizers and pesticides to promote plant

Table 7: Assessment and ranking for their ability to function of isolate

No.	Isolate	N- fixation	P- solubility	K- solubility	IAA	GA_3	Thermo-tolerant	Chitinolytic	proteolytic	Cellulolytik	Total value	Rank
1	JMs-3	0	0	2	2	2	2	2	1	2	13	4
2	TMs-4	0	0	1	1	2	2	0	2	2	10	7
3	Be-2	0	0	1	2	2	1	0	0	1	7	10
4	BMBe-3	0	0	1	2	2	2	2	2	3	14	3
5	JBe-4	0	0	2	3	2	2	2	2	0	13	4
6	KWo-2.1	2	3	0	2	2	2	1	2	0	14	3
7	KWo-2.2	0	0	1	2	2	2	3	3	1	14	3
8	PGa-1.2	1	2	0	2	2	3	1	3	2	16	1
9	PGa-1.3	0	1	1	1	1	2	1	2	0	9	8
10	BGa-2.1	0	2	3	2	2	3	1	2	0	15	2
11	BGa-2.2	0	0	2	3	1	3	0	2	0	11	6
12	WGa-3	0	0	1	2	2	1	3	2	2	13	4
13	WGa-3.1	0	0	1	2	1	2	0	2	0	8	9
14	JSi-1	0	0	2	2	2	1	1	2	0	10	7
15	LSi-3	0	0	2	2	3	3	2	2	2	16	1
16	JSi-4	0	0	3	2	3	2	2	2	2	16	1
17	RBg-1	0	0	3	2	2	3	2	0	0	12	5

growth and production. Each isolate possesses multifunctional activity and can serve as both a biofertilizer (with the ability to fix nitrogen, solubilize phosphorus and potassium and produce plant hormones such as IAA and GA3) and a biocontrol agent (with the ability to produce chitinase, protease, cellulase and inhibit fungal growth), whether used as single rhizobacterial isolates or as a consortium of rhizobacterial isolates. Each isolate exhibits distinct capabilities, even within the same strain level. Therefore, to select the most effective isolate as a biofertilizer and biocontrol agent, it is necessary to score the potential of each isolate. The top-scoring rhizobacterial isolates for use as biofertilizers or biocontrol agents are those with a total value ranking between 1-3 (Table 7). Based on these criteria, the selected isolates are LSi-3 (*B. albus* strain MCCC 1A00365), JSi-4 (*B. cereus* IAM 12605), KWo-2.2 (*B. cereus* strain SJ37), KWo-2.1 (*B. proteolyticus* strain MCCC 1A00365), PGa-1.2 (*B. albus* strain MCCC 1A02146), BMBe-3 (*Bacillus albus* strain VIT-RPJ) dan BGa-2.1(*Bacillus proteolyticus* strain MCCC 1A00365). Although each of the selected isolates can be used as a and biocontrol agent on its own, each isolate has its strengths and weaknesses; therefore, it is recommended to create a consortium by combining the strengths of each isolate to overcome their respective weaknesses. Even isolates BGa-2.2 (*B. cereus* strain IAM 12605) and JBe-4 (*B. tropicus* strain AOA-CPS1), whose total values do not fall within the top three ranks, are worthy of inclusion in a consortium as they can produce higher levels of IAA than other isolates. Furthermore, consortiums of rhizobacteria have been shown to improve nutrient availability and N, P and K uptake and increase rice yields more than single rhizobacterial strains (Gupta *et al.* 2021).

Conclusion

Isolates LSi-3 (*B. albus* strain MCCC 1A02146), JSi-4 (*B. cereus* strain IAM 12605, PGa-1.2 (*B. albus* strain MCCC 1A02146), KW0-2.2 (*B. cereus* strain SJ37), KWo-2.1 (*B. proteolyticus* strain MCCC 1A00365), BMBe-3 (*B. albus* strain VIT-RPJ) and BGa-2.1(*B. proteolyticus* strain MCCC 1A00365) are highly promising for use as biofertilizers and biocontrol agents, either individually or in the consortium. Isolates BGa-2.2 (*B. cereus* strain IAM 12605) and JBe-4 (*B. tropicus* strain AOA-CPS1) have the potential to be used in consortia with other rhizobacteria to enhance the exudation of IAA by biofertilizers.

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Author Contributions

S, NJ, RE, SP and RA planned, data collecting, and writing of the research; EN helped in the editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

No conflicts of interest.

Data Availability

Data presented in this study will be available by request to the corresponding author.

Ethics Approval

Not applicable.

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Reference

Akinrinlola RJ, GY Yuen, RA Drijber, AO Adesemoye (2018). Evaluation of *Bacillus* strains for plant growth promotion and predictability of efficacy by *in vitro* physiological traits. *Intl J Microbiol* 2018:5686874

Asif R, R Yasmin, M Madiha, A Ambreen, M Mazhar, A Rehman, M Ahmad (2022). Phytohormones as plant growth regulators and safe brotectors against biotic and abiotic stress. In: *Plant Hormones – Recent Advance, New Perspectives Applications*, pp:1–12. Hano C (Ed.). IntechOpen, London

Bonaterra A, E Badosa, N Daranas (2022). Bacteria as biological control agents of plant diseases. *Microorganisms* 10:1759

Brown MR, JH Foster (1970). A simple diagnostic milk medium for *Pseudomonas aeruginosa*. *J Clin Pathol* 23:172–177

Chandra S, K Askari, M Kumari (2018). Optimization of Indole Acetic Acid (IAA) production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. *J Genet Eng Biotechnol* 16:581–586

Desai SA (2017). Isolation and characterization of Gibberellic Acid (GA3) producing rhizobacteria from sugarcane roots. *Biosci Discover* 8:488–494

Emtiazi G, M Pooyan, M Shamalnasab (2007). Cellulase activities in nitrogen fixing *Paenibacillus* isolated from soil in N-free media. *World J Agric Sci* 3:602–608

Gupta R, A Noureldeen, H Darwish (2021). Rhizosphere mediated growth enhancement using phosphate solubilizing rhizobacteria and their tri-calcium phosphate solubilization activity under pot culture assays in Rice (*Oryza sativa*). *Saud J Biol Sci* 28:3692–3700

Gusmiaty, M Restu, B Bachtiar, S Larekeng (2019). Gibberellin and IAA production by rhizobacteria from various private forest gibberellin and IAA production by rhizobacteria from various private forest. *IOP Conf Ser: Earth Environ Sci* 270:012018

Hasra F, E Pratiwi (2013). Isolation, characterization, and molecular identification of phosphate solubilizing bacteria from several tropical soils. *J Unila* 18:67–74

Husna M, E Pratiwi (2020). The Ability of *Bacillus* consortium to fix N_2 , solubilize phosphat and synthesize IAA fitohormon. *J Tanah Dan Iklim* 43:113–121

Jadhav H, SS Shaikh, RZ Sayyed (2017). Role of Hydrolytic Enzymes of Rhizoflora in biocontrol of fungal phytopathogens: In: *Rhizotrophs: Plant Growth Promotion to Bioremediation, Microorganisms for Sustainability*, Vol. 2, pp:183–203. Mehnaz S (Ed). Springer, Singapore

Javaid A, R Bajwa (2011). Field evaluation of effective microorganisms (EM) application for growth, nodulation and nutrition of mungbean. *Turk J Agric For* 35:443–452

Javed S, A Javaid, U Hanif, S Bahadur, S Sultana, M Shuaib, S Ali (2021). Effect of necrotrophic fungus and PGPR on the comparative histochemistry of *Vigna radiata* by using multiple microscopic techniques. *Microbiol Res Technol* 84:2737–2748

Kang SM, A Adhikari, K Lee, Y Park, R Shahzad, IJ Lee (2019). Gibberellin producing rhizobacteria Pseudomonas koreensis MU2 enhance growth of letatuce (*Lactuca sativa*) and chinese cabbage (*Brassica rapa, chinensis*). *J Microbiol Biotechnol Food Sci* 9:166–170

Kang SM, AL Khan, YH You, JH Kim, M Kamran, IJ Lee (2014). Gibberellin production by newly isolated strain *Leifsonia soli* SE134 and its potential to promote plant Growth. *J Microbiol Biotechnol* 24:106–112

Khan AR, A Mustafa, S Hyder, M Valipour, ZF Rizvi, AS Gondal, Z Yousuf, R Iqbal, U Daraz (2022). *Bacillus* spp. as Bioagents: Uses and application for sustainable agriculture. *Biology* 11:1763

Kumar A, BR Maurya, R Raghuwanshi (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocatal Agric Biotechnol* 3:121–128

Lestari P, DN Susilowati, IM Samudra, TP Priyatno, K Nugroho, W Nurarfa, I Setyawati, Y Suryadi (2017). Genetic diversity of Indole Acetic Acid-producing rhizobacteria based on 16S rRNA and amplified ribosomal DNA restriction analysis. *J Agron Biogen* 13:25–34

Li H, RK Singh, P Singh, Q Song, Y Xing (2017). Genetic diversity of nitrogen-fixing and plant growth promoting *Pseudomonas* species isolated from sugarcane rhizosphere. *Front Microbiol* 8:1268

Liu M, X Liu, BS Cheng, XL Ma, XT Lyu, X Zhao, Y Ju, Z Min (2016). Selection and evaluation of phosphate-solubilizing bacteria from grapevine rhizospheres for use as biofertilizers. *Span J Agric Res* 14:e1106

Ma M, X Jiang, Q Wang, D Guan, L Li, M Ongena, J Li (2018). Isolation and identification of PGPR strain and its effect on soybean growth and soil bacterial community composition. *Intl J Agric Biol* 20:1289–1297

Meena RS, S Kumar, R Datta, R Lal, V Vijayakumar, M Brtnicky, MP Sharma, GS Yadav, MK Jhariya, CK Jangir, SI Pathan, T Dokulilova (2020). Impact of agrochemicals on soil microbiota and management: A review. *Land* 9:34

Meliah S, AW Hardiyanti, N Haida, GA Putri, EQ Ainy (2020). Screening for *Fusarium* inhibiting bacteria isolated from monkey cup (*Nepenthes* spp.) Fluid). *J Ilmu Pert Indo* 25:627–635

Mendes R, P Andrea, E Diaz, L Lana, B Lobo (2020). Use of plant growth-promoting rhizobacteria in maize and sugarcane: Characteristics and applications. *Front Sustain Food Syst* 4:0136

Miljakovi'c D, J Marinkovi´c, S Baleševi´c-Tubi´ (2020). The Significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms* 8:1037

Mozumder P, RP Berrens (2006). Inorganic fertilizer use and biodiversity risk: An empirical investigation. *Ecol Econ* 62:538–543

Nader A, M Selim, A Afify, F Hauka (2019). Studying the ability of some bacteria isolated from Egyptian soils to fix nitrogen and solubilize phosphate, *in vitro*. *J Agric Chem Biotechnol* 10:179–182

Oo KT, TT Win, AA Khai, P Fu (2020). Isolation, screening and molecular characterization of multifunctional plant growth promoting rhizobacteria for a sustainable agriculture. *Amer J Plant Sci* 11:773–792

Pakhtunkhwa K, MN Products, U Island (2017). Indoleacetic Acid production and plant growth promoting potential of bacterial endophyites esolated from rice (*Oryza sativa* L.) seeds. *Acta Biol Hung* 68:175–186

Parab PD, RD Khandeparker, BD Shenoy, J Sharma (2020). Phylogenetic diversity of culturable marine bacteria from mangrove sediments of goa, India : A potential source of xylanases belonging to glycosyl hydrolase family 10. *Appl Biochem Microbiol* 56:718–728

Pirttilä AM, H Mohammad, P Tabas, N Baruah, JJ Koskimäki (2021). Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. *Microorganisms* 9:817

Poria V, A Rana, A Kumari, J Grewal, K Pranaw, S Singh (2021). Current perspectives on chitinolytic enzymes and their agro-industrial application. *Biology* 10:1319

Roca-couso R, D Flores-felix, R Rivas (2021). Mechanisms of action of microbial biocontrol agents against *Botrytis cinerea*. *J Fungi* 7:1045

Sahin F, R Çakmakçi, F Kantar (2004). Sugar beet and barley yields in relation to inoculation with N_2-fixing and phosphate solubilizing bacteria sugar beet and barley yields in relation to inoculation with N_2-fixing and phosphate solubilizing bacteria. *Plant Soil* 265:123–129

Sari UM, A Agustien, Nurmiati (2012). Screening and characterization of thermophilic cellulolytic bacteria from the Medang River Kerinci, Jambi. *J Biol Andal Univ* 1:166–171

Sembiring M, S Anwar, T Sabrina (2021). Potential test of non-symbiotic nitrogen-fixing bacteria in increasing nitrogen in andisol soil potential test of non-symbiotic nitrogen-fixing bacteria in increasing nitrogen in Andisol soil. *IOP Conf Ser: Earth Environ Sci* 782:042069

Sharf W, A Javaid, A Shoaib, IH Khan (2021). Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis. Egypt J Biol Pest Cont* 31:16

Sharma N, R Singhvi (2017). Effects of chemical fertilizers and pesticides on human health and environment: A review. *Intl J Agric Environ Biotechnol* 10:675–679

Shoaib A, H Ali, A Javaid, ZA Awan (2020). Contending charcoal rot disease of mungbean by employing biocontrol *Ochrobactrum ciceri* and zinc. *Physiol Mol Biol Plants* 26:1385–1397

Stackebrandt E, BM Goebel (1994). Taxonomic Note : A Place for DNA-DNA reassociation and 16s rRNA sequence analysis in the present species definition in bacteriology. *Intl J Syst Bacteriol* 44:846–849

Whipps JM (2001). Microbial interactions and biocontrol in the rhizosphere. *J Exp Bot* 52:487–511

Zhang C, F Konga (2014). Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. *Appl Soil Ecol* 82:18–25

Zhang J, P Wang, L Fang, Q Zhang, C Yan (2017). Isolation and characterization of phosphate-solubilizing bacteria from mushroom residues and their effect on tomato plant growth promotion. *Polish J Microbiol* 66:57–65