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Biological Activity of Indigenous Selected Plant Growth Promoting Rhizobacteria Isolates and their Ability to Improve the Growth Traits of Shallot (*Allium ascalonicum* L.)

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The bacterial leaf blight is the primary disease and causes a significant loss of shallot yield in West Sumatera, Indonesia. Plant growth-promoting rhizobacteria (PGPR) is essential in suppressing diseases and improving shallots' growth and health. This study aimed to characterize and investigate the ability of PGPR isolates to control shallots bacterial leaf blight disease and improve its growth. Ten (10) isolates of PGPR designated as *Bacillus thuringiensis* strain MRSNRZ3.1, *B. mycoides* strain MRSNUMBE2.2, *B. mycoides* strain MRBPBT2.1, *B. waihenstephanensis* strain MRBTLL3.2, *B. subtilis* strain MRTDUMBE3.2.1, *B. cereus* strain MRDKBTE1.3, *B. cereus* MRPLUMBE1.3, *Achromobacter insolitus* strain MRBPUMBE1.3, *Pseudomonas hibiscicola* strain MRTLDRZ2.2, and *Bacillus* sp. strain MRSPRZ1.1 were collected from the rhizosphere and endosphere of shallots from Agam and Solok Regency. The variables observed were biocontrol characters (HCN production and siderophore) and biofertilizer characters (indole acetic acid production, ammonia, and phosphate dissolution). These isolates showed activity in controlling leaf blight caused by *Xanthomonas axonopodis*. The present study also suggests that PGPR isolates, *viz.* MRSNRZ3.1, MRSNUMBE2.2, MRBPBT2.1, MRBTLL3.2, MRTDUMMBE3.2.1, MRDKBTE1.3, MRPLUMBE1.3, MRBPUMBE1.3, MRTLDRZ2.2, and MRSPRZ1.1 may be used as biofertilizers to enhance the growth and productivity of shallots. This finding suggests that identified PGPR isolates can be used as biological control agents and biofertilizers to enhance the growth and productivity of shallot.

Keywords: ammonia solubilization, HCN, IAA, PGPR, phosphate solubilization

INTRODUCTION

Shallots (*Allium ascalonicum* L.) are high economic value horticultural crops, widely used mainly as kitchen spices, industrial raw materials, and medicines (Yanti *et al.* 2018). The production of shallots in West Sumatra from 2017–2019 was 95,534, 113,865, and 122,399 tons, but the production is still relatively low compared to the shallot's national production, which reached 1,580,247 tons (BPS Provinsi Sumatera Barat 2020). One of the causes of the low output of shallots is the attack of disease-causing pathogens – namely, bacterial leaf blight, an important disease in shallots, especially in West Sumatra (Yanti 2015).

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Efforts to control pathogens that have been carried out are cultural practices, physical, mechanical, genetic, and chemical, but they are not yet adequate (Pandey *et al.* 2016). Therefore, it is necessary to carry out an environmentally friendly control effort such as biological control using PGPR (plant growth promoting rhizobacteria). PGPR plays a vital role in supporting and increasing plant resistance and growth.

Most of the bacteria associated with plants come from the soil. Some bacteria can move into the rhizosphere and rhizoplane before showing a beneficial effect on the host plant. Some rhizoplane colonizing bacteria can also penetrate plant roots. Some strains may move to aerial plant parts, with a decreasing bacterial density compared to rhizosphere or root colonizing populations (Compant *et al.* 2010).

Based on the colonization area, bacteria are grouped into the rhizosphere (around the roots), rhizoplane (root surface), and endophytes. Endophytic bacteria are bacteria that not only inhabit the rhizosphere or rhizoplane but can also enter plant tissues and colonize other plant parts such as roots, shoots, and seeds without causing damage to the host plant. Many endophytic bacteria increase plant growth (Hallmann 2001; Compant *et al*. 2010). These bacteria can increase germination, biomass, leaf area, chlorophyll, nitrogen, protein, hydraulic activity, root and shoot length, and resistance to abiotic stresses such as drought, humidity, and salinity. Plants have been colonized by various endophytes, most of which originate from the rhizosphere and have been used to improve plant growth. After the rhizosphere, endophytes can also colonize multiple plant parts (James *et al*. 2002; Compant *et al*. 2005).

PGPR can replace the use of chemical fertilizers, pesticides, and supplements. Some PGPR, especially inoculated on seeds before planting, can colonize plant roots. PGPR is a component in reducing agrochemicals and is a biocontrol agent. The integrated system can be used on vegetable crops to produce plants more tolerant of nematodes and other diseases for at least a few weeks after transplanting into the field (Kloepper *et al*. 2004).

In recent years, the number of PGPR identified has increased substantially, mainly because the role of the rhizosphere and endosphere as an ecosystem is increasingly important in the biosphere's functioning. Various bacterial strains include *Aeromonas*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Klebsiella*, *Pseudomonas*, and *Serratia* have been identified as PGPR in the rhizome and plant endosphere (Podile and Kishore 2006). Some PGPR from bacterial strains such as *Bacillus* sp., *Microbacterium* sp., *Pseudomonas* sp., *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Burkholderia ambifaria*, *Azotobacter chroococcum*, *Klebsiella oxytoca*, and *Rhizobium pusense* have been studied explicitly concerning onions (Afify *et al*. 2018; Samayoa *et al*. 2020; Pellegrini *et al*. 2021).

The utilization of bacteria in agricultural production depends on our knowledge of bacterial plant interactions and our ability to maintain, manipulate, and modify beneficial bacterial populations under field conditions (Hallmann 2001). The study of plant-associated bacteria is essential for understanding the ecological role in their interactions with plants and for the biotechnology application of these bacteria to fields such as plant growth promotion (de Souza *et al.* 2015; Massa *et al*. 2022). This study aimed to characterize bacterial isolates obtained from the rhizosphere and endosphere of shallots and select and identify the isolates that can suppress the pathogen that causes onion blight (*Xanthomonas axonopodis* pv. *alii*) and can stimulate the growth of shallot plants.

MATERIALS AND METHODS

Isolation and Characterization

Plant and soil samples were taken from Solok Regency and Agam Regency, West Sumatra Province, Indonesia using purposive sampling. The samples' criteria were healthy shallot plant roots aged 6–10 wk around shallot plants with symptoms of bacterial leaf blight. Soil samples taken as much as 100–150 g were put into plastic bags, then the samples were taken to the laboratory and used in bacterial isolation. Plant samples included roots, stems, and bulbs. Soil samples were taken from the rhizosphere soil. Bacterial strains were isolated from the rhizosphere (using the dilution method), root endophytes, stems, and bulbs of onions using King's B media (containing protease peptone 20 g/L, dipotassium hydrogen phosphate 1.5 g/L, magnesium sulfate heptahydrate 1.5 g/L, and agar 15 g/L) for *Pseudomonas* and nutrient agar (meat extract 3 g/L, meat peptone 10 g/L, and agar 15 g/L) for other bacteria. The isolated strains were characterized biochemically and physiologically – biochemical and physiological tests conducted according to methods by Yanti *et al*. (2020), Sutariati *et al.* (2021), and Yavarian *et al*. (2021). Bacterial isolates were characterized molecularly by partial sequencing of the 16S rDNA gene. All selected isolates were stored in micro tubes filled with sterile water at 4 °C for further testing.

PGPR Selection

The red onion bulbs of the Bima variety were cut in 1/3 parts and surface sterilized by soaking each in 5% sodium hypochlorite for 5 min. The onion bulbs were then rinsed three times in aquadest to clean them of sodium hypochlorite

before introducing bacteria. A total of 48 bacterial isolates were grown on each medium and incubated on a shaker at 150 rpm at a temperature of 28 ± 2 °C for 48 h. The sterilized shallot bulbs were treated with a suspension of 48 bacterial strains using 1% carboxymethyl cellulose (CMC) as a carrier for 15 min and air-dried for 20 min. Shallot bulbs were planted in polybags with a volume of 10 kg containing a mixture of soil and manure (1:5), which had been sterilized by steaming for 1 h. Plants were inoculated 14 d after planting by injuring (by pricking) plant leaves and sprayed with *Xanthomonas axonopodis* pv. *alii* 10^8 CFU/mL suspension using a hand sprayer. The Suspension was compared with mac Farlaand 10^8 scale. After 80 d, the onion plants are harvested. The growth observation indicators at the selection stage were the tubers' fresh and dry weight. Onion bulbs were dried in an oven at 65 °C for 3 d to obtain the dry weight of the bulbs, whereas the indicators of plant disease were incubation period, incidence, and severity of the disease. The incubation period was observed every day until the first symptoms appeared. The disease incidence was observed 1 wk after inoculation at once a week. A week after the first symptoms appeared, the disease severity was observed once a week.

Identification of Bacteria Based on the 16S rRNA Gene Sequence

DNA extraction. DNA was extracted according to the Purelink Genomic DNA Mini Kit (Invitrogen) protocol. Pellets of bacteria were suspended with 180 L Purelink Genomic Digestion Buffer solution, added 20 L Proteinase K, and homogenized by the vortex to lyse Gram-negative bacteria cells. Bacterial samples were incubated at 55 °C in the oven and homogenized every 10 min until the lysis process was complete (30 min). 20 L of RNase A was added to the lysate, homogenized by vortex, and incubated at room temperature for 2 min. 200 L of Purelink Genomic Lysis/Binding Buffer was added to the lysate and vortexed, then 200 L of 96–100% ethanol was added to the lysate and homogenized for 5 s. The bacterial pellet was resuspended for Gram-positive bacteria with 180 L of lysozyme digestion buffer, homogenized by vortex, and incubated at 37 °C for 30 min. A total of 20 L Proteinase K was added, homogenized, then 200 L PureLink Genomic lysis/binding buffer, homogenized, and incubated again at 55 °C for 30 min in the oven. 96–100% ethanol 200 L was added to the lysate and homogenized for 5 s. The DNA binding, washing, and elution were carried out according to the Purelink Genomic DNA Mini Kit (Invitrogen) protocol (Yanti *et al.* 2021).

DNA amplification by polymerase chain reaction (PCR). DNA was amplified with primer 27F (5' AGA GTT TGA TCM TGG CTC AG'3) and primer 1492R (5'CGG TTA CCT TGT TAC GAC TT'3). The PCR was carried out according to the GreenTaq PCR Master Mix (Thermo Scientific) protocol. The reaction mixture was prepared according to the protocol for each reaction volume of 25 L with the mix for each reaction – namely, master mix 12.5 L, primer forward 1 L, primer reverse 1 L, and nuclease free water 7.5 L. 25 L. The PCR reaction mixture was put into 100-L microtubes, and three L DNA template samples were added to each tube. The initial conditions of the PCR were set at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 3 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. Final extension at the temperature of 72 °C for 10 min. Amplificon was electrophoresed using 1% agarose gel in 1x TAE buffer, visualized with gel imaging, and a baseband measuring + 1,500 bp was obtained. Sequencing was carried out at First Base Singapore (Yanti *et al.* 2021).

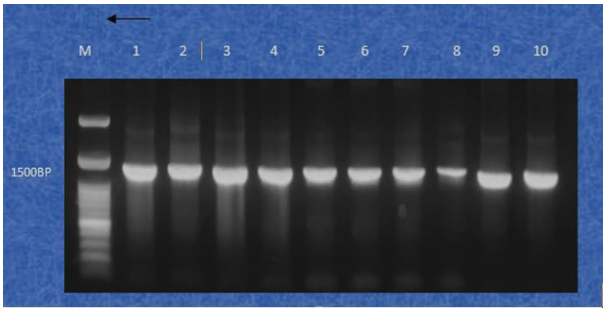

Figure 1. Amplification of the 16S rRNA gene from PGPR isolates using primers 27 F and 1387 F.

Screening of Indole Acetic Acid (IAA) Producer Strains

One mL of bacterial culture was inoculated into a 250-mL Erlenmeyer containing 100 mL of nutritious broth (beef extract 1 g/L, peptone 5 g/L, sodium chloride 5 g/L, and yeast extract 2 g/L) enriched with L-tryptophan (0.1 mg/mL) and incubated on a shaker for 48 h at 30 °C at 150 rpm. Then the bacterial cultures were centrifuged at 10,000 rpm for 30 min in the dark. Then, 2 mL of the supernatant was mixed with 2 mL of Salkowski's reagent (0.5 M FeCl3 in 35% perchloric acid) and incubated for 30 min at room temperature and in dark conditions. The optical absorbance was measured at 535 nm, and a standard curve graph measured the resulting IAA (Yavarian *et al*. 2021).

Phosphate Solubility

Bacterial isolates were screened for phosphate solubilization properties using Pikovskaya medium (containing KCl, 0.2 g L–1; MgSO4 7H2O, 0.1 g L–1; glucose, 10 g L–1; yeast extract, 0.5 g L–1; FeSO4• 7H2O, 0.002 g L–1; MnSO4•H2O, 0.002 g L–1; [NH4]2 SO4, 0.5 g L–1; Ca3[PO4]2, 5 g L–1). Bacterial strains were inserted into the middle of the medium plates using sterilized tips. Plates were incubated at 30°C for 7 d to monitor a clear halo's positive or negative development around the colonies (Samayoa *et al*. 2020).

Hydrogen Cyanide (HCN) Production

The method suggested by Lorck (1948) was used to test HCN production, in which about 100 mL of overnight strain culture was inoculated in a nutrient agar slant fortified with 4.4 g/L glycine. The tubes were covered with a Whatman filter paper (no. 1) previously soaked in 0.5% picric acid and 2% sodium carbonate. The test tubes were closed with parafilm tape and incubated at 28 ± 2 °C for 4 d. The color change of the paper from yellow to brown indicated the production of HCN.

Ammonia Production

Bacterial isolates were tested for ammonia production in peptone solution (peptone 1 g/L and sodium chloride 4.3 g/L). The newly grown cultures were inoculated in 10 mL of peptone solution in each tube and incubated for 48–72 h at 36 ± 2 °C. Nessler's reagent (0.5 mL) was added to each tube. A brown to yellow color change is a positive test for ammonia production (Bashan and Holguin 1997).

Enzyme Activity

The protease activity of the bacterial strain was carried out according to Rodarte *et al*. (2011). Bacteria were grown on skim milk agar medium (1,000-mL distilled water containing 15 g skim milk, 0.5 g yeast extract, and 9.13 g agar) and incubated at 27 °C for 48 h. The diameter of the clear zone around the bacterial colonies was measured to determine the ability of protease enzyme production. The positive activity was detected by the appearance of a clear area around the colony.

Plant Growth Onion Seeds Introduced with PGPR to Increase Growth

The red onion bulbs of the Bima variety were cut in 1/3 parts and surface sterilized by soaking each in 5% sodium hypochlorite for 5 min. Then, it was rinsed three times in distilled water to clean it from sodium hypochlorite before introducing bacteria. Bacterial isolates were grown on each medium (King's B medium for *Pseudomonas* and nutrient agar for other isolates) and incubated on a shaker at 150 rpm and a temperature of 28 ± 2 °C for 48 h. The sterilized shallot bulbs were treated with a suspension of selected bacterial strains using 1% CMC for 15 min and air-dried for 20 min. Shallot bulbs were planted in polybags containing a mixture of soil and manure (1:5), sterilizing with a volume of 10 kg. The control treatment used onion bulbs without bacterial treatment and 10 bacterial treatments. The onion crop was watered using a bucket during treatment. After 80 d, it is harvested. The number of leaves, plant height, fresh weight of tubers, and dry weight of tubers were recorded in centimeters and grams for each plant. Onion bulbs were dried in an oven at 65 °C for 3 d to obtain the dry weight of the tubers and recorded in grams.

Data Analysis

The research was conducted in an experimental field within Agriculture Faculty, Universitas Andalas, Padang. Shallot bulbs were planted, with one bulb per polybag. This study used a randomized block design with 10 replications. The treatments consisted of positive control treatment using shallot bulbs without bacterial treatment and not inoculated, negative control using shallot bulbs without bacterial treatment, streptomycin treatment, and 48 bacterial treatments – the data analysis using SAS software version 9.1. The mean comparison was tested by Duncan's test at $P = 0.05$.

Onion Growth

The growth of shallots observed was the wet and dry weights of the bulbs. The effectiveness is calculated using the formula:

$$E = \frac{T - C}{C} \times 100\% \quad (1)$$

where: E = effectiveness, T = treatment, and C = control

Onion Bacterial Leaf Blight

The observed variables were the percentage of infected leaves (%) and the intensity of the affected leaves (%) with the formula:

$$I = \frac{n}{N} \times 100\% \quad (2)$$

where: I = incidence, n = number of affected plants, and N = number of plants observed

$$S = \frac{\sum(n \times v)}{N \times V} \times 100\% \quad (3)$$

where: S = severity, n = number of leaves from each attack category, v = scale value of each attack category, N = number of leaves observed, and V = the highest numerical value in the attack category

Table 1. Percentage of infected leaves and categories of damage to bacterial leaf blight symptoms.

Percentage of leaves affected	Damage category
0	Normal
1 > x ≥ 10%	Light
10 > x ≥ 20%	Moderate
20 > x ≥ 40%	Severe
40 > x ≥ 100%	very heavy

Raju *et al*. (2011)

RESULTS

Isolation and Characterization of PGPR

PGPR isolated from stems, roots, tubers, and root-soil of healthy shallots among plants affected by HBD in West Sumatra totaled 48 isolates. The morphological characteristics obtained were as follows: the edges of the bacterial colonies were undulate (12), curled (6), entire (23), and lobate (7). The results of the Gram test showed 45 Gram-positive bacteria and three Gram-negative bacteria. Hypersensitivity reactions in the *Mirabilis jalapa* plant obtained 48 isolates of negative HR and 48 isolates that were tested for pathogenicity, none of which were pathogenic (Table 2). The isolated PGPR isolates were then tested in planta, and from 48 isolates, only 10 isolates had the highest ability to suppress *Xanthomonas axonopodis* pv. *Alii*. The results can be seen in Table 3.

The 10 best isolates were selected for further characterization. The biochemical and physiological characteristics of isolates varied widely. Selected bacterial isolates were tested for catalase, oxidase, and indole production, growth ability at 40 °C in NB medium, gelatin hydrolysis, urease activity, spore formation, and utilization of several carbon sources according to the procedures mentioned in the methods and materials. Bacterial isolates were observed molecularly by partial gene sequencing of 16S rDNA. Their accession numbers were allocated as *Bacillus thuringiensis* strain MRSNRZ3.1, *Bacillus mycoides* strain MRSNUMBE2.2, *Bacillus mycoides* strain MRBPBT2.1, *Bacillus waihenstephanensis* strain RBTLL3.2, *Bacillus subtilis* strain MRTDUMBE3.2.1, *Bacillus cereus* strain MRDKBTE1.3, *Bacillus cereus* MRPLUMBE1.3, *Achromobacter insolitus* strain MRBPUMBE1.3, *Pseudomonas hibiscicola* strain MRTLDRZ2.2, and *Bacillus* sp. strain MRSPRZ1.1.

Table 2. Morphology, gram test, hypersensitivity (HR) reaction, and pathogenicity test of PGPR isolates.

No.	Isolate		Gram reaction	HR Reaction	Pathogenicity
	Code	Colony margin	+/–	–	–
1	MRPLUMBE1.3	Lobate	–	–	–
2	MRPLE3.7	Curled	+	–	–
3	MRPL6.2	Entire	+	–	–
4	MRTLDRZ.2.2	Entire	–	–	–
5	MRLDE1.4	Undulate	+	–	–
6	MRSPRZ.1.1	Undulate	+	–	–
7	MRSNE2.1	Entire	+	–	–
8	MRDKE2.5	Entire	+	–	–
9	MRDAE1.2	Entire	+	–	–
10	MRTADE2.5	Undulate	+	–	–
11	MRTPE1.9	Entire	+	–	–
12	MRTP2E2.1	Entire	+	–	–
13	MRTP1E2.2	Undulate	+	–	–
14	MRTP1E2.1	Entire	+	–	–
15	MRTP1E1.2	Undulate	+	–	–
16	MRTP1E1.1	Lobate	+	–	–
17	MRTP2E2.2	Curled	+	–	–
18	MRTP4E1.1	Entire	+	–	–
19	MRTL3E1.1	Entire	+	–	–
20	MRDKBTE1.3	Lobate	+	–	–
21	MRTLE2.2	Curled	+	–	–
22	MRTLE2.1	Entire	+	–	–
23	MRTDUMBE 3.2.1	Entire	+	–	–
24	MRTLE1.2	Undulate	+	–	–
25	MRADE2.3	Undulate	+	–	–
26	MRTLE2.2	Entire	+	–	–

Table 2 Cont.

27	MRTLE2.1	Entire	+	–	–
28	RBTLL3.3	Entire	+	–	–
29	MRRZLL3.5	Undulate	+	–	–
30	MRBPE1.4	Entire	+	–	–
31	MRBTLL3.1	Entire	+	–	–
32	MRRD1E2	Undulate	+	–	–
33	MRRD2E1	Entire	+	–	–
34	MRBTLL3.2	Undulate	+	–	–
35	MRJB1E1	Lobate	+	–	–
36	MRJB1E2	Curled	+	–	–
37	MRJB1E3	Entire	+	–	–
38	MRSNRZ3.1	Lobate	+	–	–
39	MRSNUMBE2.2	Curled	+	–	–
40	MRBPUMBE.1.3	Entire	–	–	–
41	MRBRUMBE1.1	Entire	+	–	–
42	MRBRUMBE1.2	Undulate	+	–	–
43	MRBRUMBE2.1	Entire	+	–	–
44	MRBRUMBE2.2	Undulate	+	–	–
45	MRAPBT1.1	Lobate	+	–	–
46	MRAPBT1.2	Curled	+	–	–
47	MRAPRZ1.1	Entire	+	–	–
48	MRAPRZ1.2	Lobate	+	–	–

Table 3. The incubation period, incidence, and severity of bacterial leaf blight on shallots introduced by PGPR.

No.	Isolate code	Disease						Resistance reaction
		Incubation period	Effectiveness (%)	Incidence	Effectiveness (%)	Severity	Effectiveness (%)	
	MRBRUMBE1.1	8.00 a	–6.65	28.50 a	–2.51	28.88 a	0.00	Vulnerable
	MRBRUMBE1.2	8.00 a	–6.65	28.50 a	–2.51	28.88 a	0.00	Vulnerable
	MRBRUMBE2.1	8.00 a	–6.65	28.50 a	–2.51	28.88 a	0.00	Vulnerable
	MRBRUMBE2.2	8.57 a	0.00	28.00 a	–0.71	28.88 a	0.00	Vulnerable
	MRAPBT1.1	8.57 a	0.00	28.00 a	–0.71	28.88 a	0.00	Vulnerable
	MRAPBT1.2	8.57 a	0.00	28.00 a	–0.71	28.88 a	0.00	Vulnerable
	MRAPRZ1.1	8.57 a	0.00	28.00 a	–0.71	28.88 a	0.00	Vulnerable
	MRAPRZ1.2	8.57 a	0.00	27.90 a	–0.35	28.88 a	0.00	Vulnerable
	CONTROL -	8.57 a	0.00	27.82 a	0.00	28.88 a	0.00	Vulnerable
	CONTROL +	9.00 a	5.02	25.26 ab	9.21	21.98 ab	23.90	Quite resistant
	MRPL5.2	9.00 a	5.02	25.15 ab	9.60	21.52 ab	25.48	Quite resistant
	MRBPE1.4	9.00 a	5.02	25.09 ab	9.81	20.69 ab	28.35	Quite resistant
	MRLDE1.4	10.00 a	16.69	24.75 ab	11.04	19.89 ab	31.12	Quite resistant
	Streptomycin	10.00 a	16.69	24.50 ab	11.93	19.3 ab	33.17	Quite resistant
	MRSNE2.1	10.00 a	16.69	24.45 ab	12.11	18.9 ab	34.55	Quite resistant
	MRDKE2.5	10.00 a	16.69	24.30 ab	12.65	18.71 ab	35.21	Quite resistant

Table 3 Cont.

MRAE1.2	10.00 a	16.69	24.00 ab	13.73	18.49 ab	35.97	Quite resistant
MRTADE2.5	10.00 a	16.69	23.95 ab	13.91	18.49 ab	35.97	Quite resistant
MRTPE1.9	10.00 a	16.69	23.95 ab	13.91	18.49 ab	35.97	Quite resistant
MRTP2E2.1	10.00 a	16.69	23.75 ab	14.62	17.98 ab	37.74	Quite resistant
MRTP1E2.2	10.00 a	16.69	22.89 ab	17.72	17.89 ab	38.05	Quite resistant
MRTP1E2.1	10.00 a	16.69	22.56 ab	18.90	17.66 ab	32.40	Quite resistant
MRTP1E1.2	10.00 a	16.69	22.05 ab	20.74	17.25 ab	40.27	Quite resistant
MRTP1E1.1	10.00 a	16.69	21.90 ab	21.27	17.12 ab	40.72	Quite resistant
MRTP2E2.2	10.00 a	16.69	21.60 ab	22.35	16.98 ab	41.20	Quite resistant
MRTP4E1.1	10.00 a	16.69	21.55 ab	22.53	16.84 ab	41.68	Quite resistant
MRTL3E1.1	10.00 a	16.69	21.20 ab	23.79	16.45 ab	43.04	Quite resistant
MRDKBTE1.3	10.00 a	16.69	20.09 ab	27.78	16.09 ab	44.28	Quite resistant
MRTLE2.2	10.00 a	16.69	20.09 ab	27.78	15.88 ab	45.01	Quite resistant
MRTLE2.1	10.00 a	16.69	19.75 ab	29.01	15.72 ab	45.56	Quite resistant
MRPLE.3.1	10.00 a	16.69	19.25 ab	30.80	15.46 ab	46.46	Quite resistant
MRTLE1.2	10.00 a	16.69	19.09 ab	31.38	15.31 ab	46.98	Quite resistant
MRADE2.3	10.00 a	16.69	19.07 ab	31.45	15.11 ab	47.68	Quite resistant
MRTLE2.2	10.00 a	16.69	19.00 ab	31.71	14.96 ab	48.19	Quite resistant
MRTLE2.1	10.00 a	16.69	18.45 ab	33.68	14.79 ab	48.78	Quite resistant
MRJB1E1	10.00 a	16.69	17.09 ab	38.56	14.66 ab	49.23	Quite resistant
MRJB1E2	10.00 a	16.69	16.88 ab	39.32	14.25 ab	50.65	Quite resistant
MRJB1E3	10.00 a	16.69	16.65 ab	40.15	14.00 ab	51.52	Quite resistant
MRRDE3.9	12.00 ab	40.03	16.51 ab	40.65	13.99 ab	51.55	Quite resistant
MRRDE2.1	12.00 ab	40.03	16.12 ab	42.05	13.89 ab	51.90	Quite resistant
MRRDE1.5	12.00 ab	40.03	15.75 b	43.38	13.05 b	54.81	Quite resistant
MRSPRZ.1.1	18.25 c	112.96	10.92 c	60.74	10.28 c	64.40	Resistant
MRTLDRZ.2.2	18.25 c	112.96	10.85 c	60.99	10.21 c	64.64	Resistant
MRDKBTE1.3	18.25 c	112.96	10.60 c	61.89	10.14 c	64.88	Resistant
MRPLUMBE1.3	18.25 c	112.96	10.12 c	63.62	10.05 c	65.20	Resistant
MRSNRZ3.1	18.25 c	112.96	9.72 c	65.06	9.90 c	65.72	Resistant
MRSNUMBE2.2	18.25 c	112.96	9.50 c	65.85	9.27 c	67.90	Resistant
MRBPUMBE.1.3	19.00 c	121.70	9.50 c	65.85	8.99 c	68.87	Resistant
MRBPBT2.1	19.00 c	121.70	9.35 c	66.39	7.99 c	72.33	Resistant
RBTLL3.2	19.77 c	130.68	8.45 c	69.62	7.95 c	72.47	Resistant
MRTDUMBE 3.2.1	20.05 c	133.95	8.00 c	71.24	6.75 c	76.63	Resistant

Table 4. The wet and dry weight of shallot bulbs after the introduction of PGPR.

No.	Code isolate	Wet weight (g)	Effectiveness (%)	The dry weight of tubers (g)	Effectiveness (%)
1.	MRTDUMBE 3.2.1	125.00a	733.33	63.98 a	433.16
2.	MRSNUMBE2.2	108.88 a	625.86	63.00 a	425.00
3.	MRBPUMBE.1.3	102.25 a	581.66	60.00 a	400.00
4.	MRBPBT2.1	99.98 a	566.53	59.98 a	399.83
5.	RBTLL3.2	98.00 a	553.33	58.60 a	388.33
6.	MRSPRZ.1.1	70.89 b	372.60	38.98 b	224.83
7.	MRTLDRZ.2.2	61.56 bc	310.40	36.75 bc	206.25
8.	MRDKBTE1.3	55.69 bcd	271.26	33.98 bcd	183.16
9.	MRPLE.3.1	52.98 cde	253.20	30.88 bcd	157.33
10.	MRSPRZ.1.1	51.93 cde	246.20	29.55 bcd	146.25
11.	MRTPE 1.9	45.69 cde	204.60	28.49 bcd	137.41
12.	MRTP2E2.1	41.78 cde	178.53	27.14 bcd	126.16
13.	MRTP1E2.2	39.64 cde	164.26	26.70 bcd	122.50
14.	MRTP1E2.1	35.61 cdef	137.40	26.55 bcd	121.25
15.	MRTP1E1.2	35.21 cdef	134.73	25.88 bcd	115.66
16.	MRTP1E1.1	35.13 cdef	134.20	25.12 bcd	109.33
17.	MRTP2E2.2	35.13 cdef	134.20	24.78 bcd	106.50
18.	MRTP4E1.1	34.60 cdef	130.66	24.22 bcd	101.83
19.	MRTL3E1.1	34.20 cdef	128.00	24.09 bcd	100.75
20.	MRDKBTE1.3	33.00 cde	120.00	20.99 d	74.91
21.	MRTLE2.2	32.69 cdef	117.93	19.61 de	63.41
22.	MRTLE2.1	32.25 cde	115.00	19.55 de	62.91
23.	MRTDUMBE 3.2.1	32.00 cde	113.33	19.25 de	60.41
24.	MRTLE1.2	31.89 cdef	112.60	19.00 de	58.33
25.	MRADE2.3	31.00 cde	106.66	18.98 de	58.16
26.	MRTLE2.2	30.65 cde	104.33	18.45 de	53.75
27.	MRTLE2.1	29.98 cde	99.86	18.24 de	52.00
28.	MRJB1E2	29.64 cde	97.60	18.00 de	50.00
29.	MRJB1E1	29.58 cde	97.20	17.95 de	49.58
30.	MRJB1E3	29.20 cde	94.66	17.45 de	45.41
31.	MRRDE3.9	28.60 cde	90.66	17.20 de	43.33
32.	MRRDE2.1	28.22 cde	88.13	16.98 de	41.50
33.	MRRDE1.5	27.65 cdef	84.33	16.75 de	39.58
34.	MRSNE2.1	27.00 cdef	80.00	16.24 de	35.33
35.	MRDKE2.5	26.87 cdef	79.13	16.00 de	33.33
36.	MRDAE1.2	26.21 cde	74.73	15.89 de	32.41
37.	MRTADE2.5	25.89 cde	72.60	15.25 de	27.08
38.	MRLDE1.4	24.00 e	60.00	15.00 e	25.00
39.	Streptomycin	18.00 ef	20.00	14.75 ef	22.91
40.	MRPL5.2	18.29 ef	21.93	14.55 ef	21.25
41.	MRBPE1.4	18.35 ef	2.33	14.00 ef	16.66
42.	CONTROL +	18.26 ef	21.73	13.98 ef	16.50

Table 4 Cont.

43.	CONTROL –	15.00 f	0.00	12.00 f	0.00
44.	MRBRUMBE1.1	14.87 f	–0.86	11.87 f	–1.08
45.	MRBRUMBE1.2	14.87 f	–0.86	11.87 f	–1.08
46.	MRBRUMBE2.1	14.58 f	–2.86	11.58 f	–3.50
47.	MRBRUMBE2.2	15.00 f	0.00	12.00 f	0.00
48.	MRAPBT1.1	14.58 f	–2.86	11.58 f	–3.50
49.	MRAPBT1.2	14.77 f	-1.53	11.77 f	1.91
50.	MRAPRZ1.1	14.58 f	–2.86	11.58 f	–3.50
51.	MRAPRZ1.2	15.00 f	0.00	12.00 f	0.00

Table 5. Identification of native 16S rRNA PGPR using BLAST.

Isolate	Total base	Sequence analysis result	Percentage of similarity (%)
MRSNRZ3.1	1250	*Bacillus thuringiensis* strain ATCC 10792	100
MRSNUMBE2.2	1225	*Bacillus mycoides* strain ATCC 6462	100
MRBPUMBE.1.3	1220	*Achromobacter insolitus*	100
MRBPBT2.1	1220	*Bacillus mycoides* strain ATCC 6462	100
RBTLL3.2	1216	*Bacillus weihenstephanensis* strain ATCC 6457	99
MRTDUMBE 3.2.1	1216	*Bacillus subtilis* BSn5	99
MRDKBTE1.3	1195	*Bacillus cereus* strain RSA21	99
MRPLUMBE1.3	1195	*Bacillus cereus* strain RSA21	99
MRTLDRZ.2.2	1195	*Pseudomonas hibiscicola*	99
MRSPRZ.1.1	1195	*Bacillus sp.* RD_AZPVI_03	99

Table 6. Description of bacterial isolates.

Source	Isolate codes	Location	Coordinate	Plant parts
Field	MRSNRZ.3.1	Sungai Nanam, Solok District	1°02’55.6’’S 100°45’27.9’’E	Rhizosphere
Field	MRSNUMBE.2.2	Sungai Nanam, Solok District	1°02’55.6’’S 100°45’27.9’’E	Bulbs (endophyte)
Field	MRBPBT.2.1	Batu Palano, Agam District	0°19’46’’S 100°22’30’’E	Stem (endophyte)
Field	MRBTLL.3.2	Taluak, Solok District	1°03’51.1’’S 100°45’41.1’’E	Stem (endophyte)
Field	MRTDUMBE.3.2.1	Taluak Dalam, Agam District	0°23’10’’S 100°24’13’’E	Bulbs (endophyte)
Field	MRDKBTE.1.3	Danau Kembar, Solok District	1°02’46.8’’S 100°44’01.1’’E	Stem (endophyte)
Field	MRPLUMBE.1.3	Padang Lua, Agam District	0°20’30’’S 100°23’00’’E	Bulbs (endophyte)
Field	MRBPUMBE.1.3	Batu Palano, Agam District	1°02’55.6’’S 100°45’27.9’’E	Bulbs (endophyte)
Field	MRTLDRZ.2.2	Taluak Dalam, Agam District	1°02’55.6’’S 100°45’27.9’’E	Rhizosphere (endophyte)
Field	MRSPRZ.1.1	Simpang, Solok District	0°19’46’’S 100°22’30’’E	Rhizosphere

Table 7. Characterization of bacterial isolates.

Character	MRSNRZ 3.1	MRSNUMBE 2.2	MRBPBT 2.1	RBTLL 3.2	MRTDUMBE 3.2.1	MRDKBTE 1.3	MRPLUMBE 1.3	MRBPUMBE 1..3	MRTLDRZ 2.2	MRSPRZ 1.1
Gram reaction	+	+	+	+	+	+	–	–	–	+
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	–	–	+
Grow up to 40 ºC	+	+	+	+	+	+	+	–	+	+
Indole production	–	–	–	–	–	–	+	+	+	–
Methyl red reaction	–	–	–	–	–	–	+	+	–	–
Vogesposkauer	–	–	–	–	–	–	+	+	–	–
Spore formed	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	–	–	+	+
Fluorescent on KB	–	–	–	–	–	–	–	–	+	–
Growth aerobically	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	–	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+
Utilization of citrate	+	+	+	+	+	+	+	+	+	+
Melonate	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	–	+

Activities to Increase Plant Growth

The bacterial strains can act as plant growth-promoting bacteria because, in addition to producing HCN and IAA, they also produce siderophores. Plant growth-promoting attributes and different enzymatic activities of the bacterial isolates can be seen in Table 8.

IAA Production

All observed bacterial isolates produced IAA. The ability of bacteria to produce IAA in the rhizosphere and endosphere depends on plants' availability of precursors and uptake of microbial IAA. The appearance of pink color indicates IAA production. After qualitative analysis, these isolates were subjected to quantitative production of IAA. The results showed that the range of IAA production was 30.9–58.9 µg/mL. Among all bacterial isolates, MRBPBT2.1 produced the highest concentration of IAA (58.9 µg/mL), and the lowest IAA was found in MRSPRZ11 (30.9 µg/mL). These bacterial isolates can be a significant plant growth driving factor.

Phosphate Solubility

Piskovskaya agar medium was used to detect phosphate solubilizing microorganisms. All bacterial isolates were screened for phosphate solubilization on modified PVK agar, wherein the isolates showed various phosphate solubilizing activities. Seven isolates were positive for phosphate dissolution – namely, MRSNRZ3.1, MRSNUMBE2.2, MRBPBT2.1, MRPLUMBE1.3, MRBPUMBE1.3, MRTLDRZ2.2, and MRSPRZ1.1. Meanwhile, three other isolates – namely, RBTLL3.2, MRTDUMBE3.2.1, and MRDKBTE1.3 – showed negative results for phosphate solubilization.

Hydrogen Cyanide (HCN) Production

HCN is a dangerous chemical produced by some plant growth-promoting bacteria because it has toxic properties. All isolates were tested for HCN production. The development of yellow to reddish-brown color indicates a positive result. All isolates were positive. The isolates

MRSNRZ3.1, MRSNUMBE2.2, MRBPBT2.1, and MRB TLL3.2 showed strong results.

Ammonia Production

Ammonia production is another important property of plant growth-promoting bacteria, indirectly affecting plant growth. This study's bacterial isolates showed positive ammonia production results except for MRB TLL3.2, MRTDUMBE3.2.1, MRDKBTE1.3, and MRSPRZ1.1.

Enzyme Activity

All the results of the different enzymatic activities of the isolates are summarized in Table 8. It should be noted that each of the ten tested strains had at least three positive actions, but it was clear that the strains MRSNRZ3.1, MRSNUMBE2.2, and MRBPBT2.1 had solid enzymatic activity.

Shallot Seed Growth Introduced with PGPR to Increase Growth

All bacterial isolates were tested to determine their effect on leaf number, plant height, fresh tuber weight, and dry tuber weight (Table 9). PGPR isolates had a significant impact on the amount of leek. The results showed that the number of leaves increased in plants treated with PGPR compared to controls not inoculated with PGPR. The highest number of leaves (45.05) was recorded in the MRSPRZ1.1 isolate treatment, followed by MRTLDRZ2.2 (44.00), MRBPUMBE1.3 (39.65), MRPLUMBE1.3 (39.57), MRDKBTE1.3 (39.50), MRTDUMBE3.2.1(39.08), RBTLL3. 2 (39.00), MRSNUMBE2.2 (38.08), and MRSNRZ3.1 (38.35). Bacterial isolates significantly increased shallot plant height ranging from 40.05–57.05 cm compared to the control treatment with a plant height of 35.00 cm. The fresh weight of onion bulbs increased from 17.02–28.09 g compared to the control treatment of 15.00 g; the dry weight fiber of shallot bulbs went from 11.06–18.00 g compared to the control 9.09 g. The data obtained indicated that the PGPR treatment had a significant role in spurring the growth of shallot plants.

DISCUSSION

In this study, beneficial bacteria were isolated from the rhizosphere and endosphere of bulbs, stems, and roots of shallot plants in the field. The isolated bacteria were selected for further testing for different plant growth-promoting activities and characterized by physiological and biochemical tests. The 10 best isolates in suppressing the development of the pathogen *Xanthomonas axonopodis* pv. *alii* and increasing onion

Table 8. Plant growth-promoting attributes and different enzymatic activities of the bacterial isolates

Character	MRSNRZ 3.1	MRSNUMBE 2.2	MRBPBT 2.1	RBTLL 3.2	MRTDUMBE 3.2.1	MRDKBTE 1.3	MRPLUMBE 1.3	MRBPUMBE 1..3	MRTLDRZ 2.2	MRSPRZ 1.1
HCN production	++++	++++	++++	++++	+++	+++	++	++	++	++
IAA production (ug/mL)	46.8	42.5	58.9	41.3	36.57	36.72	36.54	34.64	36.57	30.9
P-solubilization activity	+	+	+	–	–	–	+	+	+	+
Ammonia production	+	+	+	–	–	–	+	+	+	–
Siderophore production	+	+	+	+	+	+	+	+	+	+
Similarity (%)	100	100	100	99	99	99	99	99	99	99

Table 9. Plant growth-promoting effects of the isolated bacterial on shallots plant.

Treatment	Number of leaves	Plant height (cm)	Fresh weight of tubers (g)	Tuber dry weight (g)
Control	30.00 ± 1.63 a	35.00 ±2.06 a	15.00 ± 0.82 a	9.09 ± 0.05
MRSNRZ 3.1	38.35 ± 2.16 b	40.05 ± 0.71 b	17.02 ± 0.50 a	11.06 ± 0.98
MRSNUMBE 2.2	38.08 ± 4.32 b	42.08 ± 2.45 b	20.05 ± 0.48 b	13.09 ± 0.82
MRBPBT 2.1	39.00 ± 0.82 b	44.00 ± 2.45 b	20.08 ± 1.28 b	13.00 ± 0.82
RBTLL 3.2	39.00 ± 1.63 b	49.00 ± 1.63 c	20.05 ± 1.41 b	15.00 ± 0.82
MRTDUMBE 3.2.1	39.08 ± 4.76 b	52.00 ± 1.41 c	25.06 ± 1.63 c	15.09 ± 1.17
MRDKBTE 1.3	39.50 ± 0.36 b	53.00 ± 0.82 c	25.09 ± 0.82 c	15.70 ± 1.46
MRPLUMBE 1.3	39.57 ± 1.23 b	53.05 ± 1.63 c	27.00 ± 0.82 c	17.96 ± 1.49
MRBPUMBE 1..3	39.65 ± 4.77 b	55.00 ± 1.71 c	25.09 ± 0.82 c	16.95 ± 1.14
MRTLDRZ 2.2	44.00 ± 1.26 c	55.00 ±2.83 c	27.26 ± 0.83 c	17.06 ± 0.82
MRSPRZ 1.1	45.05 ± 2.16 c	57.05 ± 1.41 c	28.09 ± 0.98 c	18.00 ± 0.82

*Mean (± SD) followed by the same letter within a column are not significantly different at $p \leq 0.05$.

growth were MRSNRZ 3.1, MRSNUMBE 2.2, MRBPBT 2.1, BTLL 3.2, MRTDUMBE 3.2.1, MRDKBTE 1.3, MRPLUMBE 1.3, MRBPUMBE 1.3, MRTLDRZ 2.2, and MRSPRZ 1.1. All bacterial isolates were positive for IAA production. The bacterial isolate MRBPBT2.1 produced a high concentration of IAA (58.9 µg/mL), followed by MRSNRZ3.1 (46.8 µg/mL), and the lowest concentration of IAA was found in MRSPRZ11 (30.9 µg/mL). It has been reported in previous studies that IAA production by plant growth-promoting bacteria may vary among different species and strains and is also influenced by culture conditions, growth stage, and substrate availability (Mirza *et al*. 2001).

Plant growth-promoting bacteria facilitate plant growth directly by acquiring resources (nitrogen, phosphorus, and essential minerals) or regulating plant hormone levels, or indirectly by reducing the inhibitory effects of various pathogens on plant growth and development in the form of biocontrol agents (Yanti *et al*. 2020).

The exact mechanism to stimulate plant growth by PGPR is not detected. However, several hypotheses – such as suppression of damaging organisms, phosphate solubilization, production of phytohormones such as IAA, and increased absorption of mineral nutrients – are usually believed to be involved (Glick 2012; Lalande *et al*. 1989).

Seven of the 10 isolates could solubilize phosphate and produce sharp phosphate dissolution zones on PVK agar media. Ammonia production is another critical aspect of PGPR to stimulate plant growth. The bacterial isolates in this study produced ammonia except for MRBTLL3.2, MRTDUMBE3.2.1, MRDKBTE1.3, and MRSPRZ1.1. HCN production was detected in all isolates – namely, MRSNRZ3.1, MRSNUMBE2.2, MRBPBT2.1, and MRBTLL3.2 – showed robust results; whereas other isolates – namely, MRTDUMBE3.2.1, MRDKBTE1.3, MRPLUMBE1.3, MRBPUMBE1.3, MRTLDRZ2.2, and MRSPRZ1.1 – showed intermediate results. Many soil microorganisms produce HCN production, and HCN plays a role in biologically controlling pathogens (Mohite 2013).

The results showed that PGPR could increase the growth of shallot plants. Ten (10) PGPR isolates were observed as the most efficient for increasing the number of leaves and plant height and fresh weight and dry weight of shallot bulbs, which were indicated by the observations above the control treatment. In general, the results obtained from this study showed that PGPR isolated from the rhizosphere and endosphere of shallots could induce IAA production and dissolution of phosphorus plus increase plant growth. Using PGPR as a biofertilizer inoculant efficiently replaces chemical fertilizers and pesticides for sustainable shallot cultivation. Further investigations, including efficiency tests under field conditions, are needed to clarify the role of PGPR as a biofertilizer that has beneficial effects on plant growth and development.

CONCLUSION

The results showed that from 48 rhizobacteria isolates, only 10 isolates had the highest ability to suppress *Xanthomonas axonopodis* pv. *alii*. PGPR was able to increase the growth of shallot plants. All PGPR isolates were observed as the most efficient for increasing the number of leaves and plant height and fresh weight and dry weight of shallot bulbs, indicated by the observations above the control treatment results. The PGPR isolated from the rhizosphere and endosphere of shallots could

induce IAA production and phosphorus dissolution plus and increase plant growth. As a biofertilizer inoculant, PGPR can replace chemical fertilizers and pesticides for sustainable shallot cultivation. Further investigations, including efficiency tests under field conditions, are needed to clarify the role of PGPR as a biofertilizer that benefits plant growth and development.

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