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Evaluation of *Bacillus cereus* bioinoculants for biocontrol of bacterial leaf blight and growth promotion in shallots

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[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman. Jl. DR. Soeparno No. 61, Purwokerto, Banyumas 53122, Central Java, Indonesia
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Abstract. *Prihatiningsih N, Arifianto RA, Istiqomah D, Irwandhi. 2026. Evaluation of Bacillus cereus bioinoculants for biocontrol of bacterial leaf blight and growth promotion in shallots. Asian J Agric 10 (1): g100136. https://doi.org/10.13057/asianjagric/g100136.* Bacterial leaf blight (*Xanthomonas axonopodis* pv. *allii*) is a major disease of shallots (*Allium cepa*) that requires continuous control. The use of rhizobacterial bioinoculants is an environmentally friendly strategy that serves a dual role, as a biocontrol and a plant growth promoter. This study aims to evaluate the bioinoculant *Bacillus cereus* in controlling bacterial leaf blight and increasing shallot growth. This research was conducted using a factorial Randomized Block Design (RBD) with 2 factors (cropping pattern and biofertilizer) and 3 replications. All *B. cereus* treatments inhibited the pathogen's growth in vitro via a bacteriostatic mechanism, with isolate Bm3 producing the largest inhibition zone (14.75 mm). In addition, each *B. cereus* isolate can produce siderophores and proteases, as well as dissolve phosphate. In field tests, *B. cereus* treatment can significantly reduce disease development compared to the control. The consortium treatment tended to show the highest efficacy, with low disease intensity (8.33%), control effectiveness of 51.93%, reduced AUDPC (23.50%), and increased nutrient uptake of shallot. These results indicate that the *B. cereus* bioinoculant, in the form of a consortium, has the potential to be an effective multifunctional inoculant for controlling bacterial leaf blight and enhancing growth in sustainable shallot cultivation.

Keywords: *Bacillus cereus*, bacterial leaf blight, shallot, *Xanthomonas axonopodis* pv. *allii*

INTRODUCTION

Bacterial leaf blight disease in shallots caused by *Xanthomonas axonopodis* pv. *allii* causes economic losses in various countries. One of the countries affected by this pathogen is Indonesia. Bacterial leaf blight of shallots has been reported to spread and infect production centers in Indonesia in Cirebon, Tegal, Nganjuk, Bantul, and Sigi, with attack severity ranging from 62.50% to 100% (Asrul et al. 2013). *Xanthomonas axonopodis* pv. *allii* attacks on shallot plants are characterized by initial symptoms in the form of water-soaked spots on the leaf tips, which cause chlorosis and necrosis until the leaves die (Yanti et al. 2025). Under suitable environmental conditions, severe *X. axonopodis* pv. *allii* attacks can reduce crop yields by 50-100% (Yanti and Hamid 2023). The level of attack risk can increase with high disease intensity, especially during the rainy season, resulting in unstable shallot crop productivity (Korlina et al. 2023). Furthermore, improper cultivation practices can exacerbate disease severity (Leiwakabessy et al. 2024). To minimize crop production losses, pathogen control measures are necessary according to severity (Gent and Schwartz 2005).

Bacterial leaf blight control is commonly carried out by some farmers using chemical methods. One such chemical control is the use of copper-based bactericides (Belo et al. 2023). Long-term chemical control can impact human health, phytotoxicity, pathogen resistance, and reduced biodiversity (Ćurković et al. 2016; du Toit et al. 2021; Istiqomah et al. 2021). This is in line with Ayilara et al. (2023), who showed that approximately 98% of chemical control used by farmers on soils impacts non-target organisms. It is necessary to develop alternative control strategies that are more environmentally friendly, effective, and support agricultural sustainability. One approach is the use of biobactericides based on the rhizosphere bacterium *Bacillus cereus*.

Rhizobacteria *B. cereus* is a Gram-positive, antagonistic bacterium that produces antibacterial compounds. Previous studies have shown that *B. cereus* is effective in controlling *Magnaporthe oryzae* (Zhou et al. 2021), *Meloidogyne incognita* (Yin et al. 2021), and *Ralstonia solanacearum* (Wang et al. 2019). This relates to the ability of *B. cereus* to induce plant defense systems against pathogens (Yanti and Nasution 2017). Inoculation of *B. cereus* into plants can increase peroxidase activity (Yanti 2015). This enzyme plays a role in strengthening cell walls and inhibiting pathogen infection (Prasannath 2017). *Bacillus cereus* can produce salicylic acid at around 13.96-14.72 ppm mL^{-1} and produce antibiotics with an inhibition zone against *X. axonopodis* pv. *allii* of up to 16.25 mm (Resti et al. 2018). Furthermore, the microencapsulated formulation of *B.*

cereus strain C1L was also effective in controlling lily leaf blight, with results comparable to those of chemical pesticides (Chen et al. 2013). Furthermore, this bacterium also has the potential to be used as a plant growth-promoting agent (biofertilizer).

It has been reported that *B. cereus* can stimulate plant growth through biological mechanisms. *B. cereus* plays a role in increasing nutrient availability, soil health, and stimulating plant metabolism (Hassan 2017; Basu et al. 2021). This strain produces indole-3-acetic acid (IAA), protease, ACC deaminase, β-1,3-glucanase, amylase, cellulase, and can solubilize phosphate and potassium minerals (Ku et al. 2018; Ali et al. 2021; Zhou et al. 2021). In addition, *B. cereus* also produces hydrogen cyanide (HCN), ammonia, and siderophores that increase iron availability (Kumar et al. 2020; Sherpa et al. 2021). However, reports on *B. cereus* formulations as biofertilizers and biocontrol agents against *X. axonopodis* pv. *alli,* combined with shallot cultivation systems, is still very limited, and to the best of our knowledge, this is the first report. Most previous studies have focused on *B. cereus* formulations that increase shelf life and field effectiveness, inoculation timing, varietal interactions, and mechanisms of growth enhancement (Martínez-Álvarez et al. 2016; Zhou et al. 2021; Pratiwi et al. 2024; Kurniawan et al. 2026). This study aims to evaluate the bioinoculant *B. cereus* in controlling bacterial leaf blight and increasing shallot growth. Developing a dual-function *B. cereus* bioinoculant (biocontrol and biofertilizer) could be an innovative strategy for controlling bacterial leaf blight while sustainably increasing shallot productivity.

MATERIALS AND METHODS

Location

This research was conducted in the period April to August 2025. The experimental field of Linggasari Village, Kembaran Sub-district, Banyumas District, Central Java, Indonesia (7°23'47.03"S-109°15'57.30"E; 119 m above mean sea level). This research location has an average temperature of 24-32°C, rainfall >2,000 mm per year, high relative humidity (70-80%), and a sunshine duration of 12 hours per day. The analysis of the potential of *B. cereus* Bm1, Bm2, Bm3, Bm4, and the consortium as biocontrol in vitro, took place at the Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas District.

Preparation of *B. cereus* isolate suspension and *X. axonopodis* pv. *allii* isolation

Bacillus cereus isolate used in this biofertilizer formulation was taken from the cultures preserved during the previous research by Saputra et al. (2024), which was isolated from the rhizosphere of healthy shallots (*Allium cepa*) with accession number SUB14858200 NPBM1 PQ578645, SUB14858200 NPBM2 PQ578646, SUB14858200 NPBM3 PQ578647, and SUB14858200 NPBM4 PQ578648. Isolation was carried out by inoculating it into 100 mL of Nutrient Broth (NB) media and shaking at 150 rpm at room temperature for 24 hours (Prihatiningsih et al. 2022). The consortium was prepared by mixing 20 mL of each *B. cereus* isolate suspension. *Xanthomonas axonopodis* pv. *allii* isolated from plants with bacterial leaf blight, was identified through colony morphology, Gram reaction, and biochemical assays (Irwandhi et al. 2024). Pathogenicity was confirmed by Koch's postulates, fulfilling all criteria.

Enzyme activity of *X. axonopodis* pv. *allii*

Pectinase and cellulase production

Pectinase and cellulase tests were performed qualitatively by observing the formation of a clear zone. This clear zone is used as an indication of plant cell wall degradation. The pectinase test used Pectinase Screening Agar Medium (PSAM) (1.50 g Na_2HPO_4, 0.75 g KH_2PO_4, 0.38 g K_2HPO_4, 1.25 g NaCl, 2.5 g glucose, 1.25 g pectin, 1.88 g agar and 100 mL distilled water) which was heated and sterilized at 121°C for 15 minutes. *Xanthomonas axonopodis* pv. *allii* pathogen was inoculated into the PSAM medium using the point inoculation method. After 5 days of incubation, the medium was flooded with KI-I_2 solution for 5 minutes, and a clear zone around the colony indicated pectinase activity (Khalil et al. 2020).

Cellulase activity test was carried out using Carboxymethyl Cellulose (CMC) media (2.0 g KH_2PO_4, 1.4 g $(NH_4)_2SO_4$, 1.4 g KNO_3, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.3 g $CaCl_2$, 5.0 mg $FeSO_4 \cdot 7H_2O$, 1.6 mg $MnSO_4 \cdot H_2O$, 2.0 mg $CoCl_2$, 1.4 mg $ZnSO_4 \cdot 7H_2O$, 1 L distilled water, and 15-20 g agar) which was then sterilized and mixed at a temperature of around 50°C. The isolates were inoculated by the spot method and incubated at 28°C for 5 days. After incubation, the medium was dripped with 0.1% Congo Red for 15 minutes. The formation of a clear (yellow) zone indicates cellulase activity through CMC degradation (Ahmad et al. 2013).

Assessment of functional traits of rhizobacteria

Siderophore production

For siderophore production testing, the SD-CASA media method was used (Shin et al. 2001). The media consisted of 60.5 mg of Chrome Azurol S (CAS) dissolved in 50 mL of distilled water, then mixed with 10 mL of Fe(III) solution containing 1 mmol L^{-1} $FeCl_3 \cdot 6H_2O$ and 10 mmol L^{-1} HCl, then shaken using a shaker. Next, 72.9 mg of HDTMA dissolved in 40 mL of water was added slowly until a dark blue solution was formed, then diluted with 2000 mL of water. For solid media, 2% (w v^{-1}) agar was added and sterilized by autoclaving at 121°C for 15 minutes. After that, 10 mL of the solution was poured into a 9 cm diameter Petri dish as a basic agar medium (CASA). After hardening, the medium was coated with 6 mL of NA and incubated overnight at 32°C (Prihatiningsih et al. 2017). This process causes the release of dye and changes the color of the medium from blue to yellow-orange around the bacterial colonies (Nithyapriya and Lalitha 2019).

Phosphate solubilizing

The test of the ability of bacteria to dissolve phosphate was carried out using Pikovskaya media (10 g glucose, 5 g Ca_3HPO_4, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4$, 0.1 g

$7H_2O$, 0.5 g yeast extract, 25 mg $MnSO_4$, 25 mg $FeSO_4$, 20 g bacto agar, and 1 L distilled water) (Mukamto et al. 2015). Bacterial isolates were grown on Pikovskaya media using the double culture method, then incubated for 5-7 days. Observations were made to observe the formation of holozones (clear zones) around bacterial colonies, which indicate the ability of bacterial isolates to dissolve phosphate. Conversely, the absence of this zone indicates the opposite (Kamaluddin et al. 2025). Meanwhile, quantitative testing was done through analysis using a spectrophotometer to determine the concentration of dissolved phosphate produced (Asrul and Aryantha 2020).

Protease test

Qualitative protease production testing was performed by growing the isolate on skim milk agar medium (5 g casein, 2.5 g yeast extract, 1 g glucose, 15 g agar, 7% skim milk, and 1000 mL distilled water). Protease activity was tested using a single-loop streaking technique on the medium, and the ability of the bacteria to degrade proteins was indicated by the formation of a clear zone around the colony (Majumdar and Chakraborty 2017).

Bacterial antagonism test

Testing was conducted using a dual culture method to observe the inhibitory power of *B. cereus* Bm1, Bm2, Bm3, Bm4, and bacterial consortium against *X. axonopodis* pv. *allii* with a bacterial cell population of 10^8 CFU mL^{-1} (Balouiri et al. 2016). A 20 µL *X. axonopodis* pv. *allii* culture suspension was inoculated into NA medium using the pour method. A 10 µL *B. cereus* culture was dropped onto a 6 mm diameter paper disc, then incubated for 24 hours at room temperature to observe antagonistic activity (Figure 1).

The area of the inhibition zone was calculated using the formula: I = zone diameter - paper disc diameter (mm). Then, the antibacterial power was categorized as done by Al-Farabi et al. (2024) in Table 1. The isolate's antibiosis mechanism against *X. axonopodis* pv. *allii* was observed by taking a portion of the clear zone and placing it in a test tube containing 0.6% peptone water. The suspension was then shaken for 24 hours at 150 rpm at room temperature. The cloudy condition of the peptone water indicates that the bacterial antibiosis mechanism is bacteriostatic, while the clear peptone water indicates that the bacterial antibiosis mechanism is bactericidal (Bernatová et al. 2013). The turbidity level is used as an indirect indicator of the viability of bacterial isolates and does not quantitatively confirm the number of bacterial cells.

Plant experiments

The factorial Randomized Complete Block Design (RCBD) during plant experiment, with treatments planting pattern (M: Monoculture, T: Intercropping) as the first factor and *B. cereus* isolate type in biofertilizer (K, Bm1, Bm2, Bm3, Bm4, and consortium) as the second factor (Table 2). The experiment involved a total of 12 treatments, each three replications. The field experiment was conducted in Linggasari Village, Kembaran Sub-district, Banyumas District, from April to July 2025. Shallot seedlings were planted at a spacing of 15×15 cm, with one seedling per planting hole. Biofertilizer application was performed by spraying the root zone with 250 mL of biofertilizer ($\geq 10^8$ CFU mL^{-1}) diluted in 5 L of water, applied once per week. Bacterial leaf blight develops under natural field infection conditions without artificial inoculation. Disease suppression is evaluated by comparing treated plants with untreated control plants. Observed variables included incubation period, disease intensity, infection rate, AUDPC, and control effectiveness. Data were analyzed using Analysis of Variance (ANOVA). Statistical analyses were performed using IBM SPSS Statistics software (29.0.2.0 (20)). The significant differences among treatments were determined using Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Table 1. Antibacterial power categories of rhizosphere isolates against pathogen growth

Category	Inhibition zone diameter
Very strong	>20 mm
Strong	10-20 mm
Medium	5-10 mm
Weak	<5 mm

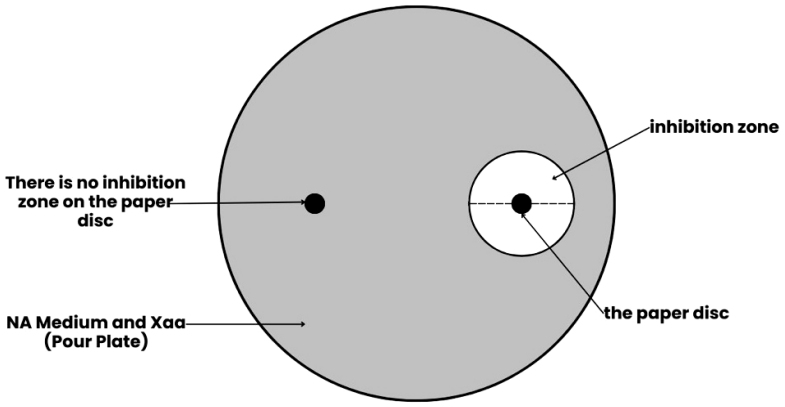

Figure 1. Measurement of inhibition zones between *Bacillus cereus* and *X. axonopodis* pv. *allii* isolates

Table 2. Biofertilizer treatment formulations applied to shallots (*A. cepa*) under different planting patterns

Code	Treatment
MK	monoculture without application of *B. cereus* biofertilizer formula
MP1	monoculture + Bm1 biofertilizer formula
MP2	monoculture + Bm2 biofertilizer formula
MP3	monoculture + Bm3 biofertilizer formula
MP4	monoculture + Bm4 biofertilizer formula
MP5	monoculture + consortium biofertilizer formula
TK	intercropping without application of *B. cereus* biofertilizer formula
TP1	intercropping + Bm1 biofertilizer formula
TP2	intercropping + Bm2 biofertilizer formula
TP3	intercropping + Bm3 biofertilizer formula
TP4	intercropping + Bm4 biofertilizer formula
TP5	intercropping + consortium biofertilizer formula

Table 3. Symptom categories in experimental plants

Category value	Symptomatic plants
0	0% (no attack)
1	1-20%
2	21-40%
3	41-60%
4	61-80%
5	81-100%

Disease intensity observation

Disease intensity observations were conducted when the plants were 2 to 6 weeks after planting (WAP). Disease intensity was calculated using the formula according to Triwidodo and Tanjung (2020).

$$DI = \frac{\sum(n \times v)}{Z \times N} \times 100\%$$

Where, DI: Disease intensity (%), n: Number of plants per symptom category, v: Symptom category score, Z: Highest score (v=5), N: Total plants observed. The category values used in this research refer to Triwidodo and Tanjung (2020) as in Table 3.

Determination of the severity of plant disease through AUDPC calculations using the Nugroho et al. (2015) formula.

$$AUDPC = \sum_{i.i}^{n} \frac{X_{(i+1)} + X_i}{2} (t_{(i+1)} - t_1)$$

Where, AUDPC: Area Under Disease Progress Curve, Xi: Disease intensity in week i, ti: Observation time in week i, n is the observation time when the disease is terminal. The effectiveness of suppressing bacterial leaf blight disease by *B. cereus* using the calculation formula according to Nugroho et al. (2015):

$$ED = \frac{ID_c - ID_t}{ID_c} \times 100\%$$

Where, ED: Effectiveness of disease suppression, ID_c: Intensity of disease control, and ID_t: Intensity of disease with treatment.

Plant nutrient uptake

Nitrogen (N), phosphate (P), and potassium (K) levels were measured when the plants reached their maximum vegetative phase, 11-35 days after planting (Hazra et al. 2021). Leaves, tubers, and roots were oven-dried, ground, and processed using the wet pulverization method (acid digestion) prior to nutrient analysis. This method involves wet pulverizing and digesting 0.5 g of dried plant samples using a concentrated acid mixture (H_2SO_4-HN_3) under controlled heating until a clear solution is obtained. The digested extract was diluted with distilled water and filtered. Nitrogen content was determined using the Kjeldahl method, phosphorus was measured colorimetrically using a spectrophotometer, and potassium was measured using Atomic Absorption Spectrophotometry (AAS).

RESULTS AND DISCUSSION

Enzim activity of *X. axonopodis* pv. *allii*

The analysis of the production capacity of pectinase and cellulase enzymes by *X. axonopodis* pv. *allii*, shown in Figure 2, gave positive results. Positive *X. axonopodis* pv. *allii* produced these enzymes, as indicated by the formation of a clear zone around the colony. This clear zone shows that the *X. axonopodis* pv. *allii* isolate can degrade pectin and cellulose. These two extracellular enzymes break down components of cell walls, pectin and cellulose, produced by *X. axonopodis* pv. *allii*. As a result, plant physical defenses become weaker. Pectinase degrades pectin, which weakens cell bonds and loosens plant tissue (Büttner and Bonas 2010; Peng et al. 2021; Obomighie et al. 2025). Cellulase enzymes, including endoglucanase, exoglucanase, and β-glucosidase, break down cellulose in plant cell walls into simple sugars (Suryaningrum and Samsudin 2019). The production of these cell wall-degrading enzymes in *X. axonopodis* is regulated by the rpoE gene (Geng et al. 2025). The combined activity of these two enzymes increases pathogen penetration and colonization in plants by weakening cell adhesion and plant tissue structure, which supports disease development (Guerriero et al. 2015; Ramos et al. 2016).

Functional traits of rhizobacteria

The functional characteristics of rhizobacteria in producing siderophores and proteases in Table 4 indicate that each isolate has distinct capabilities. All rhizobacterial isolates can produce siderophores. This ability is indicated by the formation of an orange zone around the colony on CASA media (Figure 3.A). The orange zone indicates that the isolate produces hydroxamate-type siderophores (Radhakrishnan et al. 2014). This finding is consistent with previous research showing that *B. cereus* can produce hydroxamate and catecholate siderophores (Sinha et al. 2019). The ability of bacteria to produce siderophores suggests they can stimulate plant growth. Siderophore-producing bacteria play a role in alleviating Fe deficiency and improving plant physiological and biochemical processes in saline soils (Sultana et al. 2021), under

drought conditions, and in plants experiencing heavy metal stress (Hofmann et al. 2020).

The results of the protease enzyme activity test (Table 4) show that all *B. cereus* rhizobacteria isolates produce protease enzymes with varying levels of activity. Isolate Bm2 is a bacterium with a Proteolytic Index (2.8), while Bm3 has the lowest Proteolytic Index (0.7). This indicates that each bacterium can degrade complex proteins into simpler peptides and amino acids at varying levels (Hastuti et al. 2017). The presence of a clear zone around the colony (Figure 3.B) indicates a protein-degradation process (Edlin et al. 2014). The higher the enzyme concentration produced, the more effective the bacteria are in inhibiting pathogen growth (Yuniati et al. 2015; Khairah et al. 2023). Research by Basurto-Cadena et al. (2012) and Irwandhi et al. (2024) shows that bacterial proteases exhibit antagonistic activity against pathogens.

All rhizobacterial isolates were able to dissolve P with Phosphate Solubilizing Index (PSI) values ranging from 3.02 to 5.24, which are categorized as moderate to high and not statistically significantly different (Table 5). Isolate Bm4 had the highest PSI value, at 5.24 (high category). This ability was characterized by a clear zone surrounding the bacterial colony (Figure 4). P-solubilizing ability was categorized into three levels: high, moderate, and low. These levels were determined based on the PSI values produced, which were greater than 5 mm, 2-4 mm, and 1 mm, respectively (Guardiola-Márquez et al. 2023). This P-solubilizing activity is related to organic acids, such as gluconic acid, citric acid, and oxalic acid, produced by bacteria (Sharma et al. 2013). These organic acids function to bind calcium (Ca) ions from the compound $Ca_3(PO_4)_2$, so that phosphate ions (H_2PO^{4-}) can be released and become available in dissolved form (Alfiansyah et al. 2023).

Bacterial antagonism activity

The results of the rhizobacterial antagonism test against *X. axonopodis* pv. *allii*, shown in Table 6 and Figure 5, indicate that all bacterial isolates can inhibit *X. axonopodis* pv. *allii* growth, with a strong inhibition category. However, the inhibition zone values formed between isolates are not significantly different. This indicates that each isolate has relatively similar antibacterial ability. Furthermore, Table 6 also shows that all rhizobacterial isolates inhibit the growth of *X. axonopodis* pv. *allii* by a bacteriostatic mechanism. Colony size and inhibition zone do not always reflect an isolate's antimicrobial activity, because the production and diffusion of antimicrobial compounds are influenced by various biological and physical factors (Yanti and Nasution 2017). These results are consistent with previous research that found that 11 *Bacillus* sp. isolates had an inhibition zone against *X. axonopodis* of around 10.47-23.20 mm with a bacteriostatic inhibition mechanism (Nurcahyanti et al. 2021).

Table 4. Characteristics of rhizobacteria in producing siderophores and protease enzymes

Treatment	Siderophore test	Proteolytic Index
Bm1	+	1.20bc
Bm2	+	2.81d
Bm3	+	0.70a
Bm4	+	1.55c
Consorsium	+	0.96ab

Note: + means that bacteria can produce siderophores. The numbers followed by the same letter in the plant disease intensity variable column do not show significant differences based on the 5% DMRT test

Table 5. The ability of rhizobacteria to solubilize phosphate

Treatment	Phosphate Solubilizing Index	Category
Bm1	3.58a	Medium
Bm2	3.02a	Medium
Bm3	4.92a	Medium
Bm4	5.24a	High
Consorsium	3.89a	Medium

Note: The numbers followed by the same letter in the plant disease intensity variable column do not show significant differences based on the 5% DMRT test

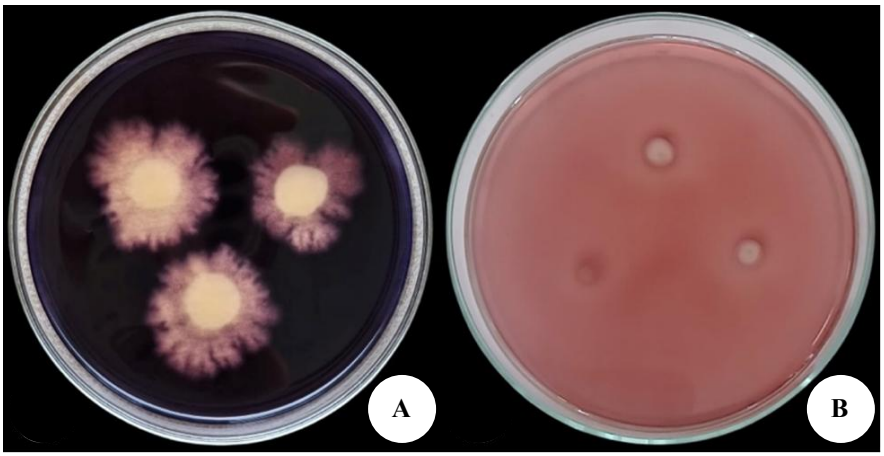

Figure 2. Pectinase test (A) and Cellulase test (B) of *X. axonopodis* pv. *allii* isolates on selective media

Table 6. Rhizobacterial antagonistic activity and inhibition mechanism against *X. axonopodis* pv. *allii*

Isolate code	Colony diameter (mm)	Inhibition zone (mm)	Inhibition zone diameter category	Inhibition mechanism
Bm1	0.88	13.50a	Strong	Bacteriostatic
Bm2	1.80	13.88a	Strong	Bacteriostatic
Bm3	0.90	14.75a	Strong	Bacteriostatic
Bm4	1.05	14.13a	Strong	Bacteriostatic
Consorsium	1.23	14.06a	Strong	Bacteriostatic

Note: The numbers followed by the same letter in the plant disease intensity variable column do not show significant differences based on the 5% DMRT test

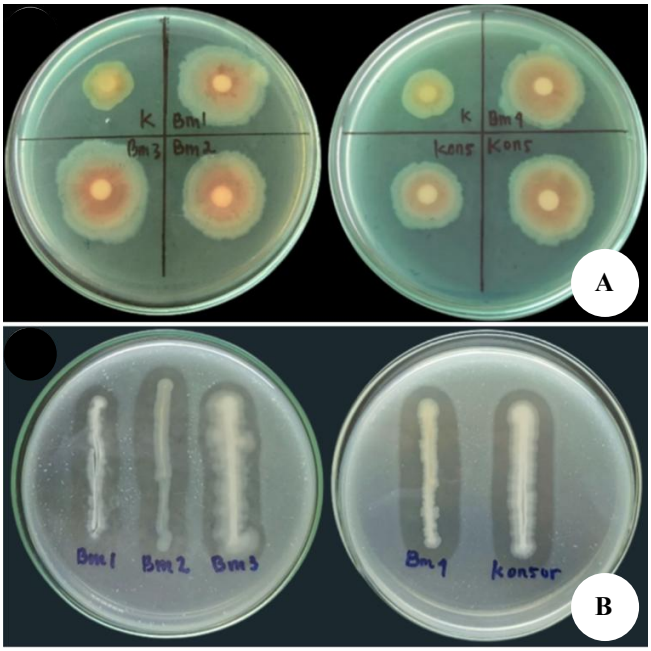

Figure 3. The ability of rhizobacteria to produce Siderophores (A) and Protease enzymes (B)

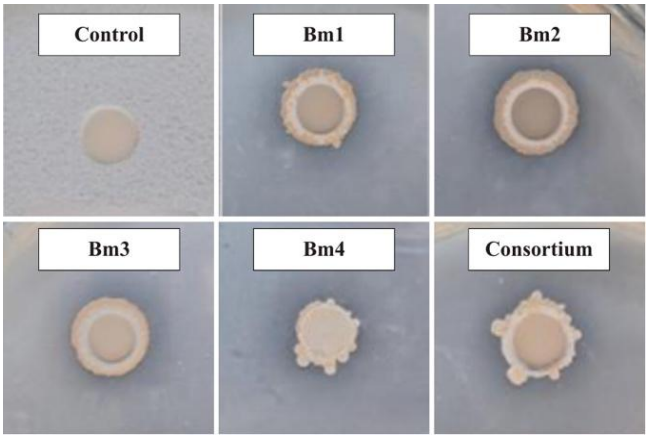

Figure 4. Formation of a clear zone around a rhizobacteria colony as an indicator of phosphate solubilization activity

Figure 5. Clear zones between *B. cereus* and *X. axonopodis* pv. *allii* isolates indicate antagonistic activity

Effectiveness of *X. axonopodis* pv. *allii* control in plants

The results of the effectiveness test of *B. cereus* rhizobacteria against *X. axonopodis* pv. *allii* in shallot plants (*A. cepa*) are shown in Table 7. The table shows that the incubation period for *X. axonopodis* pv. *allii* in all treatments was 14 days after planting. This occurs because the *B. cereus* liquid biofertilizer was applied on the 14th day after planting. This condition was done to prevent antagonistic bacteria from interacting with the pathogen in the early stages of infection. However, infection levels varied across treatments. The biofertilizer treatments tended to have lower infection rates compared to the control. These findings indicate that the application of rhizobacteria can suppress disease development by producing enzymes and antimicrobial compounds, as well as triggering systemic resistance (Ngalimat et al. 2021).

Analysis of disease intensity 35 days after planting showed no statistically significant differences. However, the consortium treatment (P5) in the monoculture (M) and intercropping (T) systems tended to have the lowest disease intensity compared to the other treatments, while the single *B. cereus* treatment also showed a decrease (compared to the control), although not yet significant. These results are in line with previous research showing that the *B. cereus* isolate B315 can inhibit pathogen growth through antibiosis mechanisms and induce systemic resistance in plants (Prihatiningsih et al. 2015). In addition, the AUDPC analysis showed that the K treatment had the highest AUDPC value of 45.33 (monoculture system) and 45.00 (intercroping system), while the lowest was observed in the P5 treatment, with 23.50. The relatively low AUDPC values in all rhizobacterial treatments compared with the

control indicate that the application of *B. cereus*, either alone or in consortium, was effective in suppressing *X. axonopodis* pv. *allii* development in shallots. Table 7 also shows that TP5 had the highest *X. axonopodis* pv. *allii* control effectiveness (51.93%). These results are included in the category of being quite capable of controlling plant diseases. The effectiveness of biological agent control is categorized into five categories, namely incapable (0%), very little capable (1-20%), less capable (20-40%), quite capable (40-60%), and very capable (>80%) (Maysixteen and Haryadi 2022). This effectiveness indicates a synergistic effect between isolates in producing antimicrobial compounds and increasing plant colonization. A combination of several antagonistic strains will be more effective than using a single isolate (Krzyzanowska et al. 2019).

Plant nutrient uptake

The results of the nutrient absorption analysis in shallot plants (Table 8) show variations in N, P, and K absorption across treatments. Although not significantly different, the application of *B. cereus* rhizobacteria tended to increase nutrient uptake in shallot plants. The treatment of Bm4 rhizobacteria in a monoculture system (MP4) tended to increase N, P, and K absorption compared to the control (MK) by 96.0%, 27.78%, and 32.96%, respectively. The MP4 treatment increased N absorption, thereby increasing nitrogen availability by dissolving organic N compounds and stimulating root growth (Sapalina et al. 2022). The increase in P nutrient absorption by shallots occurred simultaneously with the dissolution of P in the soil by rhizobacteria through the production of organic acids so that P changed from inorganic P to orthophosphate form, which is easily absorbed by plants (Husna et al. 2019; Khan et al. 2023). The availability of K in shallots influences root development, thereby improving bulb quality and plant resistance to disease (Pradana and Retno 2018). These abilities can stimulate plant growth and thus impact shallot cultivation yields. Inoculation of *B. cereus* rhizobacteria can increase the gross weight and dry weight of shallot bulbs by approximately 183.17-271.27% compared to the control (Yanti et al. 2023). A comparison between monoculture and intercropping systems shows that nutrient uptake is higher in monoculture than in intercropping. This is likely related to the interaction between shallot and bok choy root systems. The Bima Brebes shallot variety has roots approximately 14-16 cm (Hidayati et al. 2017), and bok choy has roots approximately 10-20 cm long (Amri et al. 2023). The root distribution overlaping affected on nutritional competition in the rhizosphere. This study did not directly measure root biomass, root density, or root distribution. The decrease in nutrient uptake in intercropping systems may be influenced by soil nutrient competition, but observations of root architecture and biomass are needed to confirm this hypothesis (Hermawati 2016).

Table 8. Nutrient uptake of shallot (*A. cepa* L.) in monoculture and intercropping systems

Treatment	Nutrient uptake		
	Nitrogen (g plant^{-1})	Phosphate (g plant^{-1})	Potassium (g plant^{-1})
MK	0.100a	0.018a	0.179a
MP1	0.050a	0.004a	0.036a
MP2	0.117a	0.020a	0.222a
MP3	0.100a	0.023a	0.166a
MP4	0.196a	0.023a	0.238a
MP5	0.088a	0.017a	0.156a
TK	0.088a	0.018a	0.186a
TP1	0.089a	0.016a	0.196a
TP2	0.065a	0.012a	0.131a
TP3	0.082a	0.020a	0.171a
TP4	0.087a	0.015a	0.154a
TP5	0.088a	0.021a	0.164a

Table 7. Effectiveness of in planta control of bacterial leaf blight by *B. cereus*

Treatment	Incubation period (day after planting)	Infection rate (units day^{-1})	Disease intensity (%)	AUDPC (% days)	Effectiveness (%)
MK	14	0.0060	16.67a	45.33	-
MP1	14	0.0054	14.67a	33.83	12.00
MP2	14	0.0055	15.33a	35.67	8.04
MP3	14	0.0048	13.33a	34.17	20.04
MP4	14	0.0054	15.67a	39.83	6.00
MP5	14	0.0052	14.00a	32.67	16.02
TK	14	0.0063	17.33a	45.00	-
TP1	14	0.0044	13.00a	34.00	24.99
TP2	14	0.0061	14.00a	33.00	19.22
TP3	14	0.0067	16.33a	40.33	5.77
TP4	14	0.0060	16.67a	43.33	3.81
TP5	14	0.0057	8.33a	23.50	51.93

Note: The numbers followed by the same letter in the plant disease intensity variable column do not show significant differences based on the 5% DMRT test. M: monoculture, T: intercropping, K: control (without application of *B. cereus* biofertilizer formula), P1: Bm1 biofertilizer formula treatment, P2: Bm2 biofertilizer formula treatment, P3: Bm3 biofertilizer formula treatment, P4: Bm4 biofertilizer formula treatment, P5: consortium biofertilizer formula treatment

In conclusion, all *B. cereus* rhizobacterial isolates inhibited the growth of *X. axonopodis* pv. *allii* in vitro via a bacteriostatic mechanism, with isolate Bm3 producing the largest inhibition zone (14.75 mm). In addition, each *B. cereus* isolate could produce siderophores and proteases, as well as solubilize phosphate. In field trials, *B. cereus* treatment could significantly reduce disease development compared to the control. The consortium treatment showed the highest efficacy, with low disease intensity (8.33%), control effectiveness of 51.93%, decreased AUDPC (23.50%), and increased nutrient absorption of shallots. These results indicate that the *B. cereus* bioinoculant, in the form of a consortium, is a multifunctional inoculant that tends to be effective for controlling bacterial leaf blight and enhancing growth in sustainable shallot cultivation. However, further research is needed to optimize formula stability, field-scale results, and long-term efficacy under various environmental conditions.

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