










## Enhancing Melon Growth and Nutrient Uptake with Plant-Growth-Promoting Microorganisms from *Cucumis melo*

Desti Dian Amalina<sup>1</sup>, Sudadi<sup>1\*</sup>, Ningrum Meliyani Pratama<sup>1</sup>, Widyatmani Sih Dewi<sup>1</sup>, Hadiwiyono<sup>2</sup>, Slamet Minardi<sup>1</sup>, Haruhisa Suga<sup>3</sup>

<sup>1</sup> Department of Soil Science, Sebelas Maret University, Surakarta 57126, Indonesia

<sup>2</sup> Department of Agrotechnology, Sebelas Maret University, Surakarta 57126, Indonesia

<sup>3</sup> Institute for Glyco-Core Research (iGCORE), Gifu University, Gifu 501-1196, Japan

Corresponding Author Email: [sudadi62@staff.uns.ac.id](mailto:sudadi62@staff.uns.ac.id)

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### ABSTRACT

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In an effort to mitigate the environmental impact of chemical fertilizers while promoting soil sustainability, this study investigated the potential of rhizosphere-associated microorganisms to augment nutrient uptake and growth in melon crops. Soil samples were procured from three distinct melon fields and subjected to serial dilution techniques for microbial isolation on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media. Subsequent screening identified select isolates with capabilities for nitrogen fixation, phosphorus and potassium solubilization, indole-3-acetic acid (IAA) production, and antagonism against pathogens. Two bacterial isolates, designated BS 1A and BK 1, and one fungal isolate, FW 2B, were earmarked for their plant growth-promoting microorganism (PGPM) potential. Sequence analysis revealed homology of BS 1A with *Pseudochrobactrum* sp., BK 1 with *Bacillus cereus*, and FW 2B with *Trametes polyzona*. A greenhouse experiment employing a completely randomized design evaluated the effects of these isolates on melon growth. Control treatments included a positive control (C+), receiving the recommended dosage of NPK fertilizer, and a negative control (C-), devoid of both NPK and microbial treatments. The application of *B. cereus*, *Pseudochrobactrum* sp., and *T. polyzona* was observed to significantly elevate plant biomass and nutrient acquisition, with *B. cereus* demonstrating the most pronounced effect, rivalling that of chemical fertilization. The results of this study highlight the potential of utilizing select soil microorganisms as biostimulants, which could play a significant role in increasing the productivity of melon crops, thereby supporting the advancement of sustainable agricultural methodologies.

## 1. INTRODUCTION

Melon (*Cucumis melo*) is a horticultural commodity of significant economic value, ranking fourth globally among fruits in market importance [1-3]. Celebrated for its sweet, nutrient-rich flesh, melon consumption has been linked with health benefits [4, 5]. Melon plants are grown in various regions, including tropical, subtropical, temperate, and arid areas under protected agriculture [1, 6]. Melon has a high-water demand and needs nutrient balance for optimal plant growth and development [7]. The species' predilection for high-resource inputs, however, challenges sustainable cultivation [8, 9], with intensive use of chemical inputs leading to soil microflora suppression, and environmental degradation [10-12].

The rhizosphere, a nexus of soil-root interactions, teems with microbial life, modulated by root exudates rich in organic nutrients [13, 14]. Within this intricate ecosystem, microorganisms serve as crucial agents, augmenting nutrient accessibility, endowing resistance to pathogens, and regulating plant growth [11]. Notably, certain bacterial and

fungal taxa, collectively termed plant growth-promoting microorganisms (PGPM), have garnered recognition for their contributions to enhancing agricultural productivity and fortifying crop resilience [15, 16]. These microorganisms can modulate nutritional dynamics, mitigate stress, and synthesize growth hormones such as indole-3-acetic acid (IAA) [17, 18]. Notably, genera such as *Bacillus* and *Pseudomonas* have been deployed for their phosphate solubilizing and IAA synthesizing properties [19, 20], with *Bacillus* additionally acting as a biocontrol agent [21], and *Serratia* spp. enhancing phosphate and nitrogen bioavailability [22, 23].

A persistent challenge in melon cultivation is fusarium wilt, caused by *Fusarium oxysporum* f. sp. *melonis*, a pathogen with devastating impacts on plant health and crop yields [24-26]. Biological control, leveraging antagonistic microorganisms against such pathogens, presents a sustainable alternative to synthetic pesticides, aligning with ecological management practices [27].

Previous studies have demonstrated the efficacy of soil microorganisms in bolstering melon growth and disease resistance. Bioorganic fertilizers incorporating strains of

*Paenibacillus polymyxa*, *Bacillus subtilis* strain L4, and various fungi have shown reduced wilt incidence and improved yields [28]. Furthermore, *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* have suppressed mycelial growth of FOM [29], while *Bacillus cereus* and *Pseudomonas fluorescens* have promoted growth and disease resistance in melons [30]. Additionally, *Trichoderma* spp. have been identified as plant growth stimulators and protectants against *F. oxysporum* [31]. The integration of microbial inoculants with tailored fertilizer regimes holds promise for enhancing plant growth, nutrient assimilation, and soil health.

Despite these advances, research gaps remain in the identification and application of PGPM strains endemic to the melon rhizosphere. This study, therefore, is designed to isolate and characterize PGPM from the melon rhizosphere with the potential to improve NPK uptake and stimulate melon growth. It posits that these isolates will enhance nutrient assimilation and plant development, given their roles in nutrient provision, biocontrol, and IAA production. The ultimate goal is to develop a biofertilizer that can reduce reliance on synthetic inputs, thereby fostering sustainable agricultural practices and preserving soil vitality.

## 2. MATERIAL AND METHOD

### 2.1 Sampling site

Sampling activities were conducted on farmers' melon plantations in three locations Wonogiri Regency, Sragen Regency, and Karanganyar Regency (Table 1). Isolation and isolate characterization were conducted at the Laboratory of Soil Biology and Biotechnology, Faculty of Agriculture, Sebelas Maret University. The selection of sampling locations was based on interviews that had been conducted with farmer groups and local farmers.

**Table 1.** Location of soil sampling

Location	Coordinates	Altitude (m asl)	Soil Ordo
Ngadirejo Village, Eromoko District, Wonogiri Regency	7°57'9.37" S 110°52'33.01" E	168	Vertisol
Dawungan Village, Masaran District, Sragen Regency	7°28'56.12" S 110°57'45.66" E	130	Vertisol
Sukosari Village, Jumantono District, Karanganyar Regency	7°37'50.82" S 110°56'54.52" E	195	Alfisol

The first location is located in Mojosari, Ngadirejo Village, Eromoko District, Wonogiri Regency with soil ordo Vertisols (Table 1). Sampling was done when the land was being prepared for the next melon-growing season. The cultivation system uses a non-organic farming system with an irrigation system and land is only for melon cultivation.

The second location chosen is located in Dawungan Village, Masaran District, Sragen Regency with soil ordo Vertisols (Table 1). The soil samples were conducted when the melon plant was ready to be harvested, about 2 weeks before harvest. The cultivation system is like the first location with the cropping pattern used is paddy-shallot-melon.

The third location chosen for soil sampling is located in

Sukosari Village, Jumantono District, Karanganyar Regency with soil ordo Alfisols (Table 1). Melons are cultivated by Agrimaga Farmhouse in a screen house the system used is a non-organic farming system with a drip irrigation system. Soil sampling was carried out when the plants reached the initial stage of flower emergence.

### 2.2 Soil Sampling, isolation and purification of soil microorganisms

Rhizosphere soil samples were collected from three melon fields (Table 1). Isolation was conducted using the dilution method on nutrient agar (NA) for bacterial growth and potato dextrose agar (PDA) for fungal growth. After 7 days of isolation at room temperature, colony forming unit (CFU) or viable cells/gram soil was calculated by the following formula:

$$CFU = \frac{\text{Mean plate count} \times \text{Dilution factor}}{\text{Amount of dilution}} \quad (1)$$

Morphologies of the colony were observed by Bergey's Manual Systematic Bacteriology. The selected microorganisms were then purified three times on the media to obtain pure isolates using the streak method.

### 2.3 Screening of microbial potency to provide soil nutrients, produce IAA and antagonism assay

#### 2.3.1 Screening of N-fixing ability, P and K solubilization

N-fixing ability was identified by inoculating purified microbial cultures on Jensen's N-free medium and incubating for 3 days at room temperature. Microbes that have the potential to fix N are characterized by the growth of clear colonies like water droplets on the surface medium.

P and K solubilization ability was identified by inoculating purified microbial culture on Pikovskaya medium for P and Aleksandrow medium for K and incubating 3 days at room temperature. The microorganisms were characterized by the presence of a halo zone in the media around the growing colonies. The ability of mineral solubilization is then calculated by the formula of the Solubilization Index below:

$$SI = \frac{\text{halozone diameter}}{\text{colony diameter}} \quad (2)$$

#### 2.3.2 Quantitatively assay for IAA production

The production of IAA by microorganisms was quantitatively measured using the colorimetric method by adding the Salkowski reagent (150 ml of H<sub>2</sub>SO<sub>4</sub> and 7.5 ml of 0.5 M FeCl<sub>3</sub>.6H<sub>2</sub>O in 250 ml of distilled water). Bacterial isolates were cultured on 10 ml nutrient broth (NB) and fungal isolates were cultured on 10 ml potato dextrose broth (PDB). One hundred g.ml<sup>-1</sup> L-tryptophan was added as a precursor to producing IAA. The culture was then shaken at 100 rpm for 48 h at room temperature. Then the culture was centrifuged at 5000 rpm for 30 minutes. Two ml of the supernatant was taken and put into a sterile flask, then mixed with 2 ml of Salkowski's reagent and then incubated for 60 minutes at room temperature. Next, the absorbance of the solution was measured using a spectrophotometer with λ = 530 nm and quantified in an IAA standard curve.

#### 2.3.3 Biocontrol potential against *F. oxysporum*

The biocontrol potential analysis was conducted by a bi-culture antagonistic test by placing the purified fungi isolates

and the *F. oxysporum* isolates side by side on the petri dish. The *F. oxysporum* isolates were obtained from the Laboratory of Plant Pests and Disease, Faculty of Agriculture, Gadjah Mada University, Indonesia. The media used to place the isolates was PDA and mixed PDA and NA. After the isolates were incubated for 7 days, observations were made by observing the growth direction of the isolate and *F. oxysporum*, if there is an obstructed growth. The percentage of antagonism potency was calculated using the formula below:

$$F. oxysporum \text{ inhibition\%} = \frac{(a-b)}{b} \times 100 \quad (3)$$

where,

a = diameter of *F. oxysporum* on control

b = diameter of *F. oxysporum* on bi-culture

## 2.4 Sequence analysis for selected isolate

The sequence analysis was conducted to investigate the species taxa of each potential isolate based on the molecular assay. The analysis was conducted by PT. Genetika Science Indonesia using PCR and sequencing of 16S rDNA for bacteria and rDNA ITS region for fungi. A similar sequence was obtained by BLAST search at the NCBI Database.

## 2.5 Pot experimental test for selected isolate

### 2.5.1 Inoculum formulation

Three selected isolates were then cultured until the cell density reached  $10^8$  CFU.ml<sup>-1</sup>. The liquid media were used in the form of NB for bacterial cell culture and PDB for fungal spore cultures. Each isolate was cultured in each media with a stirring speed of 180-200 rpm for 3×24 hours at room temperature and then we enumerated the cell density. The inoculant was applied before planting with a dosage of 10 ml.pot<sup>-1</sup> and twice a week with a dosage of 5 ml.pot<sup>-1</sup> using a sprayer.

### 2.5.2 Experimental design

An experiment was conducted at the Greenhouse of the Faculty of Agriculture, Sebelas Maret University to examine the impact of specific inoculum on the growth of melon and the nutrient status of the soil. The research design used a CRD with a single-factor, formulation of inoculum. The formulation consists of 7 levels, viz control+ (NPK fertilization, 35 g urea, 50 g TSP, and 40 g KCl per plant), control- (without inoculum and NPK), F1 (BK 1), F2 (BS 1A), F3 (FW 2B), F4 (BK 1 + BS 1A), F5 (BK 1 + FW 2B), F6 (BS 1A + BW 2B), and F7 (BK 1 + BS 1A + FW 2B). Each treatment was repeated 3 times. C+ was chosen to see whether the effect of the inoculated isolates could be as good as the use of NPK fertilizer and C- was selected to be a comparison when there's no treatment at all. The NPK fertilizer for C+ was applied on the day before planting and the 10th, 20th, and 35th days after planting.

### 2.5.3 Greenhouse condition

The Greenhouse of the Faculty of Agriculture Sebelas Maret University is located at 7°33'41" S and 110°51'32" E with an average daily temperature of 30-33°C and humidity of 63-78%, monitored every day. We used the soil from Entisols soil ordo. Soil initial test was conducted to analyze the characteristics of the initial soil. The results of the initial soil analysis that the soil used had a neutral pH (7.3), very low

organic C content (0.65%), medium total N in the soil (0.46%), very low available P content (0.56 ppm), and moderate exchangeable K levels (25.23 mg/100g).

### 2.5.4 Melon cultivation and growth observation

We used the "Pertawi" variety with a germination rate of 85% and a high germination vigor level. Seeding was conducted until the seedlings were ± 14 days old. Then, the seeds are transferred to polybags/planting pots.

The soil to be used is sifted so that it is smooth and there are no lumps. The soil is then sterilized to remove indigenous microbes. The dose of manure used is 20 tons.ha<sup>-1</sup>. Initial fertilizer analysis was carried out to determine the nutrient content of the manure. The results of the analysis showed a pH value of 7.71, a total N of 0.23%, a P of 0.23%, and a K of 0.59%.

The cultivation treatment for these plants involves four main steps: watering, pruning, stake installation, and pesticide spraying. Watering is done once a day either before 7 a.m. or after 4 p.m. until the soil is sufficiently moist. To promote stronger growth, pruning is performed to remove excess branches and retain only one main stem. A week after planting, bamboo stakes are installed to support the plants. We use a botanical pesticide made from crushed garlic soaked in water overnight and mixed with cooking oil. This mixture is sprayed twice a week in the morning before 7 a.m. or after 4 p.m. Plant height measurements were taken once a week until the maximum vegetative period.

### 2.5.5 Plant harvesting and nutrient uptake analysis

Plant harvesting activity was conducted after the melon plants were ± 65 days after planting. Melon plants were taken and weighed to determine the fresh weight of the plants. Melon plants were then stored in an oven at 70°C for 48 hours to determine the dry weight.

Dried whole plant tissue was ground for the analysis of nutrient uptake including, N (H<sub>2</sub>SO<sub>4</sub> wet ashes), P, and K (HNO<sub>3</sub> and HClO<sub>4</sub> wet ashes) based on the procedure from the Indonesian Soil Research Centre (2005) [32].

## 2.6 Statistical Analysis

An ANOVA analysis was performed with a confidence level of 95% to evaluate the impact of the treatment on the observed variables. If the treatment showed a significant effect, the DMRT was conducted to compare the different inoculum formulations. The analysis was carried out using the SPSS 26 software.

## 3. RESULTS

### 3.1 Abundance and diversity of soil microorganisms isolated from melon rhizosphere

Based on the study, the population of the microorganisms was different for each sampling site. Isolation on NA and PDA showed that the highest bacteria population was enumerated in Wonogiri soil ( $7.93 \times 10^5$  CFU. g<sup>-1</sup> soil) and the highest fungi population was enumerated in Karanganyar ( $5.33 \times 10^3$  CFU. g<sup>-1</sup> soil) (Table 2). The varying density of microorganisms from the three locations could be attributed to differences in planting patterns, soil types, cultivation techniques, and plant age.

**Table 2.** Population density of microbes isolated from melon rhizosphere

Location	Soil Type	Population (CFU.g <sup>-1</sup> soil)	
		Bacteria	Fungi
Wonogiri	Vertisols	7.93 × 10 <sup>5</sup>	2.83 × 10 <sup>3</sup>
Sragen	Vertisols	6.50 × 10 <sup>5</sup>	3.50 × 10 <sup>3</sup>
Karanganyar	Alfisols	4.15 × 10 <sup>5</sup>	5.33 × 10 <sup>3</sup>

We selected 10 bacterial and 10 fungal isolates based on the difference in colony morphology, such as color, shape, elevation, and margin, for screening their potency in providing soil nutrients, biocontrol agents, and producing IAA (Table 3).

**Table 3.** Colony characteristic of the selected isolate

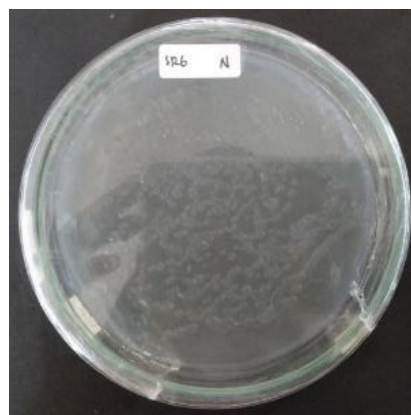
Isolate	Colony Characteristic			
	Color	Shape	Margin	Elevation
<b>Soil Bacteria</b>				
BW 1A	Off white	Irregular	Smooth	Raised
BW 1B	Off white	Irregular	Smooth	Flat
BS 1A	Off white	Irregular	Ciliate	Flat
BS 1B	Off white	Filamented	Branched	Raised
BS 2A	Off white	Round	Smooth	Raised
BS 2B	Off white	Irregular	Smooth	Flat
BK 1	Transparent	Irregular	Smooth	Flat
BK 2A	Off white	Round	Smooth	Raised
BK 2B	Off white	Irregular	Smooth	Flat
BK 3	Off white	Irregular	Smooth	Raised
<b>Soil Fungi</b>				
FW 2B	Off White	Round	Smooth	Raised
FS 1A	Off White	Round	Smooth	Raised
FS 1B	Off White	Irregular	Smooth	Raised
FS 1C	Greenish	Irregular	Smooth	Raised
FS 3A	Off White	Round	Smooth	Raised
FS 3B	Black	Round	Smooth	Raised
FK 1A	Greenish	Round	Smooth	Raised
FK 1B	Brownish	Irregular	Smooth	Raised
FK 1C	Black	Round	Smooth	Raised
FK 3A	Brownish	Round	Smooth	Raised

Note: B = Bacteria; F = Fungi; W = isolate from Wonogiri; S = isolate from Sragen; K = isolate from Karanganyar.

### 3.2 Microbial potency to provide soil nutrients

A total of 10 bacterial isolates and 10 fungal isolates were tested to identify the ability to provide soil nutrients (N, P, and K). The screening of N fixing ability was conducted by inoculating the isolates on Jensen's medium. Out of 10 bacterial isolates, it is only 1 of them had the potency for N<sub>2</sub> fixation, namely BS 1A (Table 4). It is indicated by the growth of a transparent colony on the surface of Jensen's nitrogen-free

medium (Figure 1) because the colony fixed N for its metabolisms. The microbial ability to fix N can help to enhance N uptake by plants.



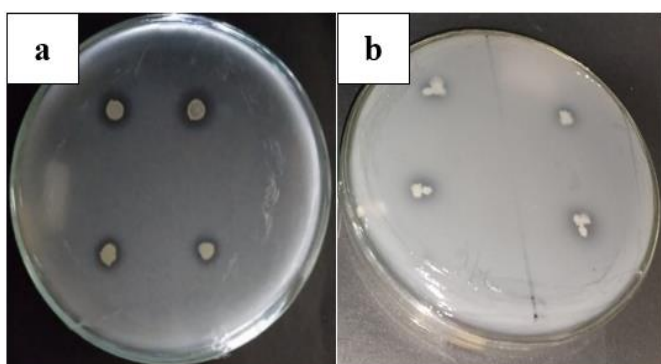
**Figure 1.** N-fixing isolate on Jensen's medium

Study results for the P solubilization assay by inoculating the isolates on Pikovskaya medium confirm that 10 bacterial isolates had P solubilization ability (Table 4) and 5 fungal isolates were positive for P solubilization ability (Table 5). The presence of a clear zone on the surface of the Pikovskaya medium indicated the ability to solubilize P. Based on the enumeration of the solubilization index, the highest P solubilization ability was shown by BS 1A with 1.96 (Table 4) and FS 3A with 1.83 (Table 5). The illustration of the halo zone is shown in Figure 2.

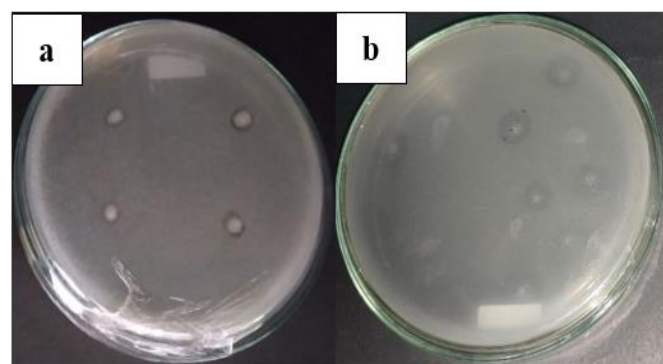
Study results on the Aleksandrow medium confirm that out of 5 bacterial isolates were positive for K solubilization ability (Table 4) and 5 fungal isolates were positive for K solubilization ability (Table 5). The appearance of a clear zone on the surface of the Aleksandrow medium indicated the ability of microorganisms to solubilize K. Based on the enumeration of the solubilization index, the highest K solubilization ability was shown by BK 2B with 1.19 (Table 4) and FW 2B with 2.79 (Table 5). The illustration of the halo zone is shown in Figure 3.

### 3.3 Microbial potency to produce IAA

In this study, L-tryptophan was used as a microbial precursor to produce IAA. The study results show the highest IAA produced by bacterial isolate BW 1B (6.15 ppm) (Table 4) and the highest IAA produced by fungal isolate FW 2B (12.99 ppm) (Table 5). The use of microorganisms that produce IAA will lead to increased plant growth.



**Figure 2.** P solubilization by BS 1A (a) and FS 3A (b)



**Figure 3.** K solubilization by BK 2B (a) and FW 2B (b)

**Table 4.** The potency of selected bacterial isolate for N<sub>2</sub> fixation, P and K solubilization, IAA production, and pathogen inhibition

Isolate	Potency to Provide Nutrients					IAA (ppm)	<i>F. oxysporum</i> Inhibition (%)
	N	P	PSI	K	KSI		
BW 1A	-	+	1.60	-	0	5.07	24.62%
BW 1B	-	+	1.41	-	0	6.15	61.54%
<b>BS 1A</b>	<b>+</b>	<b>+</b>	<b>1.96</b>	<b>-</b>	<b>0</b>	<b>5.35</b>	<b>80.77%</b>
BS 1B	-	+	1.42	+	0.88	5.38	42.31%
BS 2A	-	+	1.78	+	0.67	4.76	0%
BS 2B	-	+	1.75	-	0	4.53	230.0%
<b>BK 1</b>	<b>-</b>	<b>+</b>	<b>1.24</b>	<b>+</b>	<b>1.17</b>	<b>5.11</b>	<b>73.08%</b>
BK 2A	-	+	1.16	-	0	5.99	0%
BK 2B	-	+	1.35	+	1.20	5.02	76.92%
BK 3	-	+	1.67	-	0	5.93	20.00%

Notes: (+) = has the potency; (-) = doesn't have the potency; B = Bacteria; F = Fungi; W = isolate from Wonogiri; S = isolate from Sragen; K = isolate from Karanganyar.

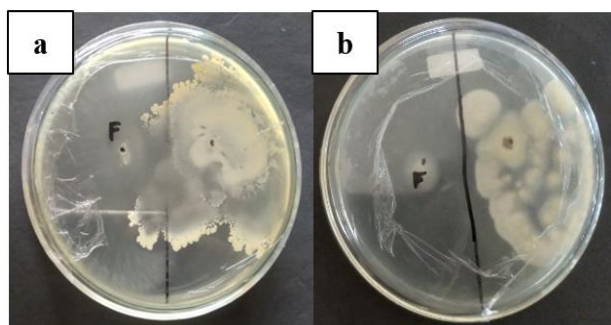
**Table 5.** The potency of selected fungal isolate for P and K solubilization, IAA production, and pathogen inhibition

Isolate	Potency to Provide Nutrients				IAA (ppm)	<i>F. oxysporum</i> Inhibition (%)
	P	PSI	K	KSI		
<b>FW 2B</b>	<b>-</b>	<b>0</b>	<b>+</b>	<b>2.79</b>	<b>12.99</b>	<b>34.62%</b>
FS 1A	+	1.19	+	1.24	6.62	26.92%
FS 1B	-	0	-	0	7.08	59.23%
FS 1C	-	0	+	1.96	9.05	30.77%
FS 3A	+	1.83	-	0	7.68	14.00%
FS 3B	-	0	-	0	9.35	42.31%
FK 1A	-	0	+	0.83	10.86	30.77%
FK 1B	-	0	-	0	12.83	0%
FK 1C	-	0	-	0	8.44	71.54%
FK 3A	+	0.84	+	0.75	9.50	0%

Notes: (+) = has the potency; (-) = doesn't have the potency; B = Bacteria; F = Fungi; W = isolate from Wonogiri; S = isolate from Sragen; K = isolate from Karanganyar.

### 3.4 Microbial potency to inhibit *F. oxysporum*

The results of the antagonist test using the bi-culture antagonist test showed that the inhibition percentage of *F. oxysporum* varied from both bacteria and fungi. The highest ability of bacterial isolates to inhibit the growth of *F. oxysporum* was shown by BS 1A (80.77%) (Table 4), and the highest ability of fungal isolate was shown by FK 1C (71.54%) (Table 5). The inhibition activity is shown in Figure 4.



**Figure 4.** *F. oxysporum* inhibition by BS 1A (a) and FK 1C (b)

### 3.5 Species identification of selected potential microorganisms for inoculum formulation

The potential microorganisms for melon growth were selected based on several characteristics based on the research.

BS 1A was selected because it is the only isolate that shows the N-fixing ability, has the highest P solubilization ability, and has the highest antagonistic potential among all the bacterial isolates. BK 1 was selected because it can solubilize P, K, and antagonistic potential. FW 2B was selected because it has the highest ability to solubilize K and the highest IAA production among all the fungi isolates (Tables 4 and 5).

Based on the sequence analysis, it is identified that BS 1A was similar to *Pseudochrobactrum* sp. with similarity (99.92%) (Table 6). BK 1 was similar to *B. cereus* with similarity (99.86%) (Table 6) and FW 2B was similar to *Trametes polyzona* with similarity (99.84%) compared to NCBI Database (Table 6).

**Table 6.** PCR results primer 16S RNA (bacteria) and ITS (fungi)

Isolate	Species	Similarity (%)	GenBank Accession Number
BK 1	<i>Bacillus cereus</i>	99.86	MN543837
BS 1A	<i>Pseudochrobactrum</i> sp.	99.92	KU986671
FW 2B	<i>Trametes Polyzona</i>	99.84	LC732073

Note: Molecular analysis of three selected isolates was carried out using 16S rDNA (bacteria) and ITS1-ITS4 (fungi) sequences, and the results were submitted to the NCBI GenBank database. The closest species and corresponding similarity percentages for each isolate were documented using the BLAST tool in NCBI.

### 3.6 The effect of PGPM formulation on plant height and biomass

The inoculation was significant due to the result of ANOVA for plant height and plant biomass, this means the application of microbe inoculum has an effect on plant height and plant biomass compared to the control (Table 7).

**Table 7.** ANOVA results for plant height and biomass

Dependent Variable	df	F	P-value
Plant height	8	10.007	0.000**
Fresh weight	8	17.278	0.000**
Dry weight	8	7.300	0.000**

Note: \*Significant at  $\alpha=0.05$ ; \*\* Highly significant at  $\alpha=0.01$ .

Treatment F6 (a combination of BS 1A and FW 2B) gives the highest result on plant height. The combination of BS 1A and FW 2B inoculum was able to increase plant height by up to 48.2% compared to the C- (Table 8). The highest plant biomass for both fresh and dry weight was F1 (single inoculant

BK 1) with an increase in plant fresh weight of up to 77.2% compared to the C- (Table 8).

**Table 8.** Results of plant height and plant biomass in greenhouse experiment

Formulation	Plant Height (cm)	Plant Biomass (gram)	
		Fresh Weight	Dry Weight
C-	195.50 <sup>a</sup>	219.00 <sup>a</sup>	47.36 <sup>ab</sup>
C+	236.83 <sup>b</sup>	380.33 <sup>d</sup>	62.92 <sup>d</sup>
F1	277.33 <sup>bc</sup>	388.00 <sup>d</sup>	63.50 <sup>d</sup>
F2	272.83 <sup>bc</sup>	255.33 <sup>ab</sup>	43.03 <sup>a</sup>
F3	274.83 <sup>bc</sup>	281.67 <sup>bc</sup>	47.96 <sup>ab</sup>
F4	267.00 <sup>bc</sup>	303.00 <sup>c</sup>	50.12 <sup>b</sup>
F5	262.33 <sup>bcd</sup>	275.00 <sup>bc</sup>	59.38 <sup>cd</sup>
F6	290.33 <sup>d</sup>	275.33 <sup>bc</sup>	56.42 <sup>c</sup>
F7	250.50 <sup>bc</sup>	226.67 <sup>a</sup>	45.21 <sup>ab</sup>

Note: The numbers followed by the same letter in the same column were not significantly different based on the DMRT test at the 5% level.

### 3.7 The effect of PGPM formulation on NPK uptake

The inoculation of microorganisms was significant due to the result of ANOVA for N, P, and K uptake, this means the microbe affects nutrient uptake ( $P < 0.05$ ) (Table 9). The highest N uptake was shown by the C+ (recommended NPK treatment) of 3.15% (Table 10). The recommended NPK treatment was able to increase plant N uptake by up to 65.6%. Meanwhile, the best inoculum formulation for increasing N uptake was shown by the F1 (single inoculum BK 1) of 2.63% (Table 10), an increase of 38% compared to the C-. This means that the application of single inoculum BK 1 can reduce the reliance on inorganic N fertilizer.

**Table 9.** ANOVA results for nutrient uptake

Dependent Variable	df	F	P-value
N uptake	8	6.110	0.001**
P uptake	8	4.950	0.002**
K uptake	8	7.792	0.000**

Note: \*Significant at  $\alpha = 0.05$ ; \*\* Highly significant at  $\alpha = 0.01$ .

**Table 10.** Results of nutrient uptake greenhouse experiment

Formulation	Nutrient Uptake (%)		
	N	P	K
C-	1,61 <sup>a</sup>	0,06 <sup>a</sup>	0,09 <sup>a</sup>
C+	2,83 <sup>bc</sup>	0,18 <sup>bc</sup>	0,26 <sup>c</sup>
F1	2,84 <sup>bc</sup>	0,17 <sup>bc</sup>	0,17 <sup>bcd</sup>
F2	3,37 <sup>c</sup>	0,13 <sup>ab</sup>	0,14 <sup>ab</sup>
F3	1,64 <sup>a</sup>	0,13 <sup>ab</sup>	0,19 <sup>bcd</sup>
F4	1,82 <sup>a</sup>	0,08 <sup>a</sup>	0,15 <sup>bc</sup>
F5	2,28 <sup>ab</sup>	0,18 <sup>bc</sup>	0,22 <sup>de</sup>
F6	3,48 <sup>c</sup>	0,17 <sup>bc</sup>	0,19 <sup>cd</sup>
F7	1,98 <sup>a</sup>	0,22 <sup>c</sup>	0,16 <sup>bcd</sup>

Note: The numbers followed by the same letter in the same column were not significantly different based on the DMRT test at the 5% level.

The highest P uptake value was shown by the F7 (combination of 3 isolates) of 0.22% (Table 10), this figure showed an increase of 244.14% compared to the control. The highest K uptake was shown by the C+ (recommended NPK treatment) of 0.26% (Table 10). The recommended NPK treatment was able to increase K uptake by up to 185.9%. Meanwhile, the best inoculum formulation in increasing K uptake was shown by treatment F5 (combination of BK 1 and FW 2B inoculums) of 0.22% (Table 10), an increase of 138.6% compared to the C-. The results mean that the use of

PGPM in melon cultivation will reduce the reliance on chemical fertilizers.

## 4. DISCUSSIONS

### 4.1 Exploration of soil microorganisms from melon rhizosphere

The isolation of microorganisms from three melon fields showed that there are various densities of microorganisms (Table 2). The melon field from Wonogiri had the highest bacterial density, while Karanganyar had the highest fungal density (Table 2). The amount of organic matter in the soil can impact the diversity and activity of microorganisms in the soil [33, 34]. These may explain the variation in colony density and morphological diversity (Table 3).

The results of the screening to provide soil nutrients, only one of the isolates found in the rhizosphere of melon plants was able to fix nitrogen, and it was identified as BS 1A (Table 3). Applying this isolate to melon growth is expected to help reduce the use of inorganic N fertilizer. Nitrogen-fixing bacteria have benefits such as increasing root surface, seed production, and fruit ripening [35]. Using a consortium of nitrogen-fixing bacteria was shown to reduce the use of inorganic fertilizers in plants by up to 25% [36].

The high P solubilization activity is expected to enhance the P availability. A higher value of PSI indicates a higher activity of phosphatase enzymes and the production of organic acids [37]. For the greenhouse experiment, PSMs BS 1A and BK 1 were selected (Table 5). The results showed that the inoculation of PSM increased the uptake of P by plants (Table 8). PSM is an alternative way to maintain soil health and minimize its degradation [38]. The use of PSM as a biological fertilizer is expected to increase the yield of fruits on melon plants, help these plants resist pathogen attacks, and reduce the excessive use of chemical fertilizers [39-41].

The K solubilization index (KSI) was used to quantify the ability to solubilize K. The results showed that BK 1 and FW 2B were suitable for inoculant application in the greenhouse experiment (Table 5). KSM produces organic acids that can solubilize K directly or chelate silicate ions first to absorb K and become available to plants [42, 43]. Utilizing KSM increased K uptake in plants (Table 8), indicating its potential as a biofertilizer. The use of KSM in agriculture as a biofertilizer can reduce the need for K fertilizer by up to 50% [44].

Plant growth is largely influenced by microbes, which produce phytohormones such as IAA that stimulate growth. IAA is a natural auxin and its production and concentration are affected by various factors such as species/strain, pH, carbon source, nitrogen source, and tryptophan availability. Microorganisms can produce varying amounts of IAA, as observed in studies using L-tryptophan as a precursor (Tables 4 and 5) [45, 46]. The use of microorganisms that can produce IAA will improve plant tolerance to pathogens by regulating growth [47]. Plants use IAA for various growth and physiological processes, including embryogenesis, organogenesis, root and shoot development, growth, and fruit development [48].

Several selected microorganisms showed the potency of *F. oxysporum* inhibition with the highest inhibition activity shown by BS 1A (Table 4) and FK 1C (Table 5). The inhibition occurs because of the mechanization of antibiotics or anti-microbials from isolates to fight pathogens [49, 50].

Several compounds synthesized by plant growth-promoting microorganisms (PGPM) such as HCN, chitinase, and gluconate make PGPM have antagonistic abilities [51]. This is expected to reduce the use of chemical products in fighting *F. oxysporum* in melon cultivation. Biocontrol agents can prevent pathogen infection, improve plant growth, and increase resistance to pathogens [21, 47].

We selected 3 potential isolates for greenhouse experiments, namely BS 1A, BK 1, and FW 2B based on the results of the screening that had been conducted. The selection was based on the outstanding characteristics of each isolate to complement each other when formulated into a biofertilizer in the future. Utilizing the PGPM with a beneficial effect for providing nutrients and plant growth offers environmentally friendly and sustainable alternatives to chemical fertilizer and disease control methods in melon cultivation.

#### 4.2 The effect of inoculation soil microorganisms on plant growth and nutrient uptake

The use of a microorganism formula on melon plants had a positive impact on their growth, as indicated in Table 7. This can be attributed to the presence of inoculum, which provides necessary nutrients for the plants [52]. The addition of inoculum leads to an increase in nutrition and subsequently, plant height [53]. Furthermore, the application of microorganism inoculum resulted in an increase in plant biomass - both fresh and dry weight (Table 8). Results from the Pearson's correlation test indicate that factors affecting plant fresh weight were N uptake ( $r=0.849^{**}$ ), K uptake ( $r=0.590^{**}$ ), and exchangeable K value in the soil ( $r=0.547^{**}$ ). On the other hand, plant dry weight was influenced by fresh weight ( $r=0.839^{**}$ ), N uptake ( $r=0.919^*$ ), P uptake ( $r=0.419^*$ ), and K uptake ( $r=0.740^{**}$ ). This demonstrates the significant impact of nutrition on plant biomass. When plants receive adequate nutrients, they can grow optimally and produce good biomass [54].

The inoculation of microorganisms that provide nutrients can effectively improve plant growth and nutrition. In this study, a single inoculation of *B. cereus* resulted in the highest increase in both fresh and dry plant biomass compared to other formulas (Table 8). *B. cereus* has been proven to promote plant growth in various studies involving sunflowers, *Phaseolus vulgaris*, and tomatoes. The ability of *B. cereus* to enhance plant growth is directly related to its production of the growth hormone IAA (Table 4). IAA is a known promoter of plant growth, and plants utilize IAA produced by microorganisms to encourage root elongation, increased root coverage, and mineral uptake, as well as root exudation [55, 56]. Furthermore, IAA contributes to plant tolerance against stress and pathogen attacks. Microbe-produced IAA enhances the number of root hair and plant lateral roots, which in turn improves plant mineral uptake and root exudation. Overall, IAA improves plant tolerance, growth, dry mass, and photosynthetic pigments [57].

The application of microorganisms in melon cultivation in greenhouses can increase NPK uptake by plants (Table 10), this is related to the ability of the applied microorganisms to fix N and dissolve P and K (Tables 4 and 5). Single inoculant *B. cereus* gave the highest N uptake results. Farmers commonly use inorganic nitrogen fertilizers to meet plant needs, but up to 65% of applied minerals are lost through gas emissions, runoff, erosion, and leaching [58]. Utilization of N-fixing bacteria (NFB) has been widely used to reduce the use

of N fertilizers in plants. NFB can convert  $N_2$  in nature into  $NH_3$  because it produces the enzyme nitrogenase. The addition of microorganisms can increase nitrogen through several mechanisms such as N fixation, enzyme activity such as N-acetylglucosaminidase and urease in soil N mineralization, ammonification, and nitrification [59, 60].

Formulation F5 which is a combination of *B. cereus* and *Trametes polyzona* gives the highest K uptake value compared to the others (Table 10). The inoculum of the two species used is known to have the ability to solubilize K (Table 10). KSM can increase the availability of K in soil solution thereby increasing the absorption of K for plant growth and development [40]. KSM can dissolve K in the soil through the mechanisms of acidolysis, chelation, exchange reactions, and complexation of the organic acids produced [14, 61, 62]. Likewise, the presence of PSM can make more P available for plant absorption [63]. PSM has been widely accepted as an environmentally friendly and easy-to-obtain P fertilizer for increasing soil orthophosphate concentrations and the geochemical P cycle since the early 20th century [41]. PSM produces organic acids and enzymes such as phosphatase and pyrophosphatase which can dissolve P to become available [64, 65]. PSM has many roles for plants, including growth boosters [39], biocontrol and bioremediation activities [40], and increasing plant growth and fruit yields [41]. The F7 formulation consisting of a combination of the three isolates, in which the three isolates could produce organic acids in dissolving K and P, was able to give the highest P absorption results.

The use of PGPM in this research plays a vital role in enhancing nutrient uptake and plant growth. This ensures the long-term health, and productivity of melon, and offers a promising path forward for more eco-friendly and efficient agricultural practices. Beyond the promotion of plant growth, PGPM also supports biocontrol and bioremediation activities, reducing the reliance on chemical products in agriculture.

#### 5. CONCLUSIONS

The application of PGPM significantly enhanced the growth of melon and NPK uptake. Inoculated plants exhibited increased plant height, fresh and dry biomass, and nutrient uptake. This underscores the importance of adequate nutrition for optimal plant development.

Among the different strains tested, only one strain, BS 1A, which was found in the melon rhizosphere, was capable of nitrogen fixation. The use of NFBs proved to be particularly effective in reducing the need for inorganic nitrogen fertilizers. Two strains, BS 1A and BK 1, were identified as effective PSMs. Moreover, BK 1 and FW 2B showed potential in solubilizing (K). The use of KSM and PSM as biofertilizers provides an eco-friendly alternative to chemical fertilizers. Among the various formulations tested, the single inoculation of *B. cereus* was the most effective in promoting plant growth and biomass production. This microorganism is known to produce the growth hormone IAA, which plays a vital role in enhancing root growth, mineral uptake, and plant tolerance to stress and pathogens.

PGPM formulations can improve melon cultivation sustainably and with minimal environmental impact. Using beneficial soil microorganisms enhances crop resilience, improves production, and promotes resource-efficient and eco-friendly farming. To determine the effectiveness of PGPM

inoculum in substituting inorganic fertilizer, further research should be conducted using different inoculum doses combined with inorganic fertilizer. This will help to reduce the use of inorganic fertilizers. Additionally, it is important to conduct resistance testing of the inoculum for field use.

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