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Assessments the synergistic effects of selected native plant growth-promoting bacteria in farmers extract organic-biofertilizer formulations for enhancing maize growth

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ABSTRACT

Farmers extract organic biofertilizer (FEOB), known as a local wisdom technology, has been widely used a significant role in demonstrating the ecological value of traditional practices in sustainable agriculture. FEOB utilize diverse microbial communities and rich in beneficial microbes, including plant growth-promoting bacteria (PGPB), to enhance nutrient bioavailability and support maize growth. This research aims to assess the synergistic interactions among three selected PGPB. The characterization and biochemical activity test were conducted to assess nitrogenase activity, production of indole acetic acid (IAA), organic acid and bioassay was conducted to evaluate the synergistic effects between the isolates. The results of the synergistic test of PGPB demonstrated a synergistic interaction between isolates N1, N3, and N6. The consortium of N136 exhibited the greatest value for plant height (30.17 cm, or approximately 68.83% higher than the control) and root length (33.33 cm, or 115.45%). Furthermore, the isolates exhibited nitrogenase activity, capable of producing IAA and organic acids, which significantly increased the chlorophyll content and biomass of maize plants by 93.62% and 94.74%, respectively, compared to the control. This finding suggests that the consortium of PGPB can be developed as an active ingredient of bioagent or biofertilizers for enhancing the agronomic characteristics of maize.

Keywords: PGPB, beneficial microbes, eco-friendly fertilizers, bioassay, native microorganisms, organic extract.

INTRODUCTION

Maize (*Zea mays* L.) is a pivotal food and industrial crop in Indonesia, playing a substantial role in national food security and the economy (FAO, 2023). Maize demand has exhibited a marked increase in recent years, attributable to population growth and the expansion of livestock industries, as it functions as both a staple food and an essential animal feed (Erenstein et al., 2022). The annual demand for maize in Indonesia exceeds 25 million tons, yet domestic production frequently falls short of meeting this demand. This discrepancy necessitates the importation of additional quantities to compensate for the shortfall. It is therefore imperative to address the issue of maize production sustainability to ensure a sufficient domestic supply and to reduce the country's reliance on imports (The Central Statistics Agency of Indonesia, 2024).

Conventional approaches, such as excessive use of chemical fertilizers, have contributed significantly to this challenge, warranting a shift towards more sustainable agricultural practices (Agric et al., 2020). The utilization of chemical fertilizers has been a multifaceted issue, exhibiting both positive and negative consequences. While these fertilizers have played a pivotal role in enhancing

agricultural productivity, their excessive use has led to substantial environmental and economic repercussions. The use of chemical fertilizers has been shown to degrade soil health, reduce microbial biodiversity, contribute to greenhouse gas emissions, and leach into water bodies, causing eutrophication (Chandini et al., 2019). In Indonesia, the environmental consequences of continuous chemical input are becoming evident, particularly in intensively cultivated areas where there is evidence of soil fertility depletion. The adverse impacts of climate change on agriculture include reduced crop yields and area, impacts on biotic and abiotic factors, economic losses, and increased labor and equipment costs (Grigorieva et al., 2023).

The global demand for ecofriendly agricultural solutions has spurred interest in alternative approaches, such as biofertilizers, which are formulated using live microorganisms capable of promoting plant growth naturally (Daniel et al., 2022). Biofertilizers represent a sustainable approach with the potential to enhance nutrient availability, soil structure, and plant resilience. In contrast to the adverse effects associated with chemical inputs, biofertilizers can promote long-term soil fertility, environmental health, and climate resilience. In this context, the focus has been on the potential contributions of native PGPB to eco-friendly agriculture. PGPB are defined as beneficial microorganisms that interact with plants in the rhizosphere, aiding growth through various mechanisms. These mechanisms include nitrogen fixation, phosphate solubilization, the production of growth-stimulating phytohormones, and the enhancement of stress resistance (Kour et al., 2020).

One of the most promising aspects of PGPB is its capacity for adaptation to local soil and climatic conditions, particularly when native strains are employed. Native PGPB isolates are inherently acclimated to regional agro-ecological systems, a trait that confers a competitive advantage over commercial strains that may encounter challenges in unfamiliar environments (Fanai et al., 2024). For Indonesian maize systems, the selection and optimization of native PGPB species could offer a sustainable solution to improving productivity and reducing chemical fertilizer dependence.

FEOB formulations are plant-based biofertilizers prepared by farmers using locally available materials, such as organic waste, plant residues, cow dung, and water. This formulation acts as a dual-purpose input for crop health by supplementing essential nutrients and enhancing microbial activity in the soil (Ammar et al., 2023). These extracts are rich in organic matter and nutrients, offering a favorable medium for inoculating PGPB and enhancing their efficacy when applied to crops. The population or growth of microorganisms within an environment can also provide insight into the interactions among them. Interspecies interactions can have a significant impact on the density of microorganisms (Anjarsari et al., 2023). Community bacteria will associate thereby increasing their effectiveness in releasing secondary metabolites compared to single isolates (Jovanita et al., 2022). The integration of PGPB into organic biofertilizer formulations, customized for specific local conditions, has the potential to yield dual benefits: an enhancement of soil health through organic inputs and an improvement in nutrient use efficiency through microbial activity (Hafez et al., 2021).

Microbial communities can influence plant growth in several ways, such as by increasing the plant's uptake of nutrients (Sodiq et al., 2019). A variety of microbes in can be used as bioactivators (Hudha et al., 2022). The Indonesian organic local microorganism (LM) of banana weevils contains biofertilizer bacteria, including PGPB such as *Aspergillus* sp., *Bacillus* sp., and *Aeromonas* sp. According to Kalay et al. (2020), the presence of rhizobacteria, including Bacillus sp. and Pseudomonas sp., has been detected in bamboo root extract. The genus Bacillus (BUX1), which is present in the rhizosphere of bamboo, has been identified as a promising source for the environmentally sound commercial production of IAA, a chemical that has been linked to the reduction of chemical pollution. The impact of the PGPB consortium formulation has been demonstrated to enhance the fresh and dry weights of maize ears. This finding indicates that the yield of maize is maximized when the consortium is utilized in comparison to a single inoculation method (Kaur et al., 2022).

The strategy under consideration is ecologically sustainable and consistent with global objectives for environmentally sound agriculture, while addressing local challenges in Indonesia. The primary objective of this study is to assess the synergistic effects of selected native PGPB when incorporated into farmers extract organic biofertilizer formulations and applied to maize cultivation. The study hypothesizes that the synergistic interactions among selected PGPB strains will not only improve maize growth parameters,

such as plant height and biomass. Biofertilizers present a more sustainable and environmentally friendly alternative to chemical fertilizers by promoting reducing ecological damage and enhanced long-term productivity. However, chemical fertilizers may still be more effective for immediate nutrient supply and short-term yield increases. An integrated approach, combining biofertilizers and reduced doses of chemical fertilizers, is often recommended to maximize benefits while minimizing environmental harm.

MATERIAL AND METHOD

Preparation of FEOB

The research study encompasses a process that commences with the formulation of the substance through anaerobic extraction methods, along with the preparation of the necessary tools and materials. The materials for this research study, mainly: bamboo roots, banana trunks, rabbit urine, goat urine, golden apple snails, tajin water (hick water from cooked rice often fed to infants as milk substitute), coconut water, molasses. The equipment utilized in this study included farmers extract organic biofertilizer formulas, such as : plastic drum (20 L capacity), small hose (1 m length), bottles (600 ml mineral water bottles) (Table 1).

The production process of farmers extract organic biofertilizer formulas commences with the precise placement of the requisite ingredients into a meticulously prepared drum, adhering strictly to the specifications detailed in the accompanying table. Following the addition of the ingredients, the mixture is subjected to thorough agitation using a mixer to achieve uniform distribution and to mitigate the formation of clumps or incomplete blending. Subsequent to mixing, the drum is hermetically sealed to establish an airtight environment, thereby precluding the ingress of external air. A strategically positioned hole is created in the drum lid to accommodate the attachment of a hose, which is subsequently inserted into a water-filled bottle. This configuration serves a dual purpose: it facilitates the expulsion of gases generated during fermentation and prevents the intrusion of external air into the drum. The mixture is then allowed to ferment over a period of three weeks, during which it undergoes biochemical transformations essential for producing a viable fertilizer extract. Upon completion of the fermentation process, the drum is unsealed and the resultant fermented product is carefully transferred into airtight containers, such as bottles, employing a dipper to ensure hygiene and precision.

Microbial diversity farmers extract organic biofertilizer formulas

The characterization process involved assessing the microbial colony population using the total plate count (TPC) method, which aims to determine the number of bacterial colonies grown on agar media (Yunita et al., 2015). Isolation and characterization of isolate PGPB from farmers extract organic biofertilizer from Majalengka, West Java, Indonesia used selective media selective media Okon and Ashby medium. Isolation and characterization have been purposed to isolate PGPB from different formulas of farmers extract organic biofertilizer formulas.

Characterization and biochemical activity

Gram staining for phenotype characterization

The characterization of nitrogen-fixing bacterial isolates involves physical and biochemical analyses to understand their properties and functions. This includes examining morphological traits, such as cell shape and size, through microscopic observation to identify features linked to nitrogen fixation. Gram staining is also performed to differentiate between Gram-positive and Gram-negative bacteria, with nitrogen-fixing bacteria typically being Gram-negative. These tests provide essential insights into the bacteria's nitrogen-fixing capabilities and their potential role in promoting plant growth.

Table 1. Composition and formulas for producing FEOB

Formulation	Formulation materials
F1	Bamboo roots 1 kg, strach water 10 L and molasses 1 L
F3	Rabbit urine 10 L, strach water 10 L and molasses 1 L
F6	Bamboo roots 1 kg, banana weevils 5 kg, rabbit urine 10 L, goat urine 10 L, golden snail 5 kg, strach water 10 L and molasses 1 L

Acetylene reduction assay

Nitrogenase test is a test that can measure nitrogenase enzyme activity using the acetylene reduction assay (ARA) method. Nitrogenase is an enzyme that plays a pivotal role in the conversion of free nitrogen in the atmosphere into ammonia (NH_3) (Susilowati, 2016). The basic principle of the ARA method is to calculate the amount of ethylene gas formed from the reduction of acetylene using gas chromatography in µmol per unit time. Bacterial isolates were grown on Okon's slant agar medium and sealed with rubber caps. The gas in the tube was taken as much as 1 mL with a microsyring and then injected with C_2H_2 acetylene gas with a comparable volume value. After that, it was incubated for 1 hour. Then, 1 mL of gas in the headspace was taken to measure the C_2H_2 ethylene concentration using gas chromatography (Hawkes, 2010). Nitrogen (40 psi), hydrogen (1.5 kgf per cm²), and air (0.5 kgf per cm²) were used as the carrier gases. The ethylene concentration in each sample was determined by referencing the area under the ethylene standard. Standard curves for ethylene were prepared with concentrations ranging from 0 µg mL^{-1} to 225 µg mL^{-1}. The chromatogram results were plotted onto the ethylene standard curve. For the experiment, 1 ml of ethylene gas (C_2H_2) was injected into each culture tube containing PGPB, followed by incubation for 1 hour. After incubation, 1 mL of gas was collected from the headspace of each culture tube and analyzed for the concentration of ethylene (C_2H_4) produced using gas chromatography.

Organic acid production test

Organic acid production testing was carried out by high performance liquid cromatogram (HPLC) method using Alliance® HPLC-e2695 Separations Module by Waters. Preparation of samples was carried out by making a culture of PGPB isolates, selected on Okon media, grown for 3 days. Organic acid standards each weighed 0.1 g with a concentration of 100 ppm. Preparation of mobile phase was done by weighing KH_2PO_4 as much as 6.8 g with pH adjustment to 2.8 (addition of phosphate solution in the form of HPO_4). The sample of PGPB isolates was poured into a 1.5 mL vial through a 0.45 µm syring filter and injected on a reversed phase HPLC column using a Grace smart RP 18 5µ column and read at λ = 210 nm (mobile phase rate of 0.7 mL min^{-1} and with a time of 10 min $injek^{-1}$).

Indole acetic acid phytohormone production test

The capacity of bacteria to produce indole-3-acetic acid (IAA) was evaluated through the rejuvenation of isolates on selective media designed for nitrogen-fixing bacteria. The bacterial isolates were prepared as a suspension of 10 mL with a cell density of 10^7 CFU mL^{-1}, in accordance with the Mac Farland standard, and incubated for a period of 1 × 24 hours. The IAA concentration was then calculated by taking the culture liquid that had been shaken and centrifuging it at 5.500 rpm for 10 minutes. The IAA concentration is calculated by taking 5 mL of the shaken culture liquid and testing the ability of the obtained supernatant to produce IAA using the calorimetry method with the addition of Salkowski reagent in a ratio of 4:1. The solution is then allowed to stand for 20 minutes, after which the absorbance is measured with a spectrophotometer at a wavelength of 535 nm (Lebrazi et al., 2020).

In-vitro test for synergistic effect

The bacteria that will be used as a consortium need to be tested for synergism. Synergistic test of selected isolates on nutrient agar (NA) media with the composition: peptone (10 g), meat extract (10 g), sodium chloride (8 g), agar (15 g), distilled water (1 L). The in vitro synergism test was carried out by growing the third isolate on Nutrient Agar (NA) media in a petri dish using the streak method (Sarkar and Chourasia, 2017) and observation 3, 5 and 7 days after inoculation (DAI). The presence of synergistic interactions among isolates is indicated by the absence of an inhibition zone in the intersection area of three isolates. This observation was further substantiated through the assessment of the compatibility of PGPB with maize in a bioassay conducted in Fahreus liquid medium.

Bioassay

Bioassay were conducted on three isolates synergis selected from different formulas of FEOB. The bioassay for nitrogen fixing bacteria (NFB) was performed by culturing the isolates on N-free Fahraeus' medium. Population density 10^8 CFU mL^{-1}, three of the isolates were inoculated in Okon medium and then incubated for 72 hours at 28 °C in a 150 rpm rotary shaker. The BISI-2 type of maize was utilized in this study. Before

treatment, the maize seeds were air dried and sterilized by washing them three times with Aquadest steril. After three days of sowing in straw paper, the maize seedlings were ready for the bioassay test. The procedure for growing maize seedlings involved using a 20 × 300 mm sterile test tube. The seeds were then planted in 100 mL test tubes containing a mixture of PGPR inoculum suspension and Fahraeus' medium, combined at a volume ratio of 1:9. The responses of maize plants observed were: (a) plant height, (b) root length, (c) chlorophyll content, and (d) plant biomass. The experiment followed a randomized block design (RBD) with 8 treatments and 4 replicates so that 32 experimental tubes were obtained. The treatments consisted of the following different isolates: control or without isolate, N1, N3, N6, N13, N16, N36 and N136.

Statistical analysis

The data obtained was entered into Microsoft Office Excel and subsequently analyzed using Analysis of Variance (ANOVA) with SPSS version 26. In the event of a significant effect, a further test was conducted using the Duncan Test (Duncan Multiple Distance Test) at a significance level of 5%.

RESULT AND DISCUSSION

Microorganism diversity FEOB Formulas

Diversity levels of FEOB in relation to microbial communities has been conducted, and the results indicate that the optimization of bacterial communities is associated with the presence of PGPB. The findings suggest that the microorganism possesses the capacity to fix nitrogen, solubilize phosphate, and produce phytohormones. PGPB has been demonstrated to facilitate indirect assistance to plants through the enhancement of nitrogen fixation or the improvement of nutrient availability in the soil (Bargaz et al., 2018). FEOB is composed of a variety of bacteria, including total bacteria and nitrogen-fixing bacteria. The subsequent table presents the findings from assessing microbial colony numbers using the total plate count (TPC) method across various FEOB formulations.

The result diversity of the microorganism community present in the various formulations of the FEOB is detailed in Table 2. The highest total populations values for total bacteria was observed in F3 and F6 formula. The highest TPC value for total phosphate solubilizing bacteria has observed in formula F3. The increased TPC values indicate the presence of microbial colonies within the respective materials. The potential for distinct microbial activities associated with the different formulations suggests that each formulation may contain PGPB. The findings indicate that the FEOB can serve as a source of PGPB. The total population of nitrogen-fixing bacteria in formulas F1, F3 and F6 was higher than that of phosphate-solubilizing bacteria. Mechanism of nitrogen fixing bacteria can also as PGPB for promote the plant growth, such as : fixing the nitrogen nutrient uptake, phytohormone production. The tests was conducted for evaluate capabilities of isolates for the activity of nitrogenase, the production of indole acetic acid (IAA), and the synthesis of organic acids.

Phenotypic characterization

The isolation process for nitrogen-fixing bacteria was conducted using two selective media: Ashby and Okon. This process resulted in six bacterial isolates, which were categorized based on their respective media of origin. The isolates obtained from the Ashby medium were designated as NA1, NA3, and NA6, while those from the Okon medium were labeled N1, N3, and N6. The isolates exhibited distinct characteristics at both macroscopic and microscopic levels. Macroscopically, the colonies specific forms and appearances, enabling differentiation

Table 2. Microbial diversity total bacteria (TB), total nitrogen fixing bacteria (TNFB) and total phosphate solubilizing bacteria (TPSB) in different formulation of FEOB

Formula	TB (x 10^9 CFU mL^{-1})	TNFB (x 10^8 CFU mL^{-1})	TPSB (x 10^8 CFU mL^{-1})
F1	1.10	6.4	1.36
F3	1.12	7.5	2.05
F6	1.62	7.2	1.92

among the bacterial types. Microscopically, the cells demonstrated unique morphologies, appearing as either oval or rod-shaped. Further analysis was conducted using Gram staining, a method employed to classify bacteria based on the structure of their cell walls. The results confirmed that most of the isolates with label NA1, NA3, NA6, N3 and N6 are Gram-negative. Characterized of Gram-negative has thin peptidoglycan layers in their cell walls and an inability to retain crystal violet dye during the staining procedure (Paray et al., 2023) (Table 3).

Biochemical activity test

Nitrogen is an essential element for plants but nitrogen cannot absorbed directly from the atmosphere. The mechanism of nitrogen binding be carried out by microorganism that have the ability to fix nitrogen from the atmosphere to increase the availability of nitrogen in the soil. Organism has a nitrogenase enzyme, that can fix nitrogen from the atmosphere with mechanism symbiotic or non-symbiotic. Activity of nitrogenase can showed the result of bacteria has capability to fix nitrogen from the atmosphere. Based on the result, three selected bacteria N1, N3 and N6 has a capability for fixed nitrogen from the atmosphere. The highest activity of nitrogenase was found in isolate N6 with the value is 1.377 $\mu M\ mL^{-1}\ g^{-1}\ h^{-1}$. The result showed value activity of nitrogenase from the N1and N3 is 0.589 and 0.516 $\mu M\ mL^{-1}\ g^{-1}\ h^{-1}$. Nitrogenase activity is indicated by the ethylene content read by the injected gas chromatography. Ethylene gas in the tube is converted to acetylene by bacteria that have nitrogense enzymes (Payá-Tormo et al., 2022).

Bacteria for the promoting the plant growth not only can have one capacity in produce of nitrogenase, but also can produce phytohormones and organic acid. The one phytohormone that can increasing the growth of plant is indole acetic acid (IAA), which can be beneficial for plant growth and development. Isolates selected through the results of screening and bioassay were tested on the ability to produce phytohormone IAA. The isolate N6 produces the highest IAA phytohormone of 1.380 ppm, then the isolate with the second highest IAA concentration value is N3 of 1.146 ppm and the lowest N1 of 1.087 ppm. Variations in IAA concentrations of various bacterial isolates are caused by differences in the growth rate of bacterial cells in each isolate. Bacterial cells develop faster can increase the synthesis of the hormone IAA (Mubarok et al., 2020).

Organic acid can increase the availability of nutrient such as phosphorus, calcium, iron, zinc and manganese in the soil. Oxalic acid is produced by fungi, bacteria, plants and animals. Oxalic acid is the most highly oxidized organic compound, which can be used as an energy source or carbon source for other organisms (Palmieri et al., 2018). Based on the results of organic acid production tests on isolates, it can be seen that PGPB have the ability to produce organic acids as presented in Table 4. Isolates of PGPB are able to produce oxalic acid with different abilities. The highest oxalic acid production was produced by isolate N1 at 5.981 ppm. Oxalic acid is a low molecular weight organic acid that is most commonly found and commonly produced by living organisms.

In-vitro test for synergistic effect

Synergistic tests between PGPB isolates were conducted qualitatively to assess compatibility among the isolates, which were subsequently applied to plants as a consortium. The synergistic effect of the PGPB isolates was evaluated in vitro

Table 3. Characteristics of PGPB

Code	Macroscopic		Microscopic	
	Colony form	Colony colour	Gram	Cell shape
NA1	Round	Red	Negative	Oval
NA3	Round	Red	Negative	Oval
NA6	Round	Red	Negative	Oval
N1	Circular	Purple	Positive	Rod-shaped
N3	Circular	Red	Negative	Rod-shaped
N6	Circular	Red	Negative	Rod-shaped

Note: Microbiology laboratory analysis results (2022).

using the streak method on NA (Nutrient Agar) medium. Microbial synergism refers to the enhanced productivity of a microbial community resulting from the metabolic interactions of one or more microorganisms (Wunderlich et al., 2023). Bacteria colonize plants and promote the growth of other beneficial microorganisms, creating a synergistic effect that promote plant growth (Tang et al., 2020) (Table 5).

The incorporation of microbial consortia requires considered as there is a risk of inhibiting the growth of one or more inoculated microorganisms. The effectiveness of biological fertilizers depends on the cooperative synergy within bacterial consortia. The bacterial isolates intended for biological testing were evaluated for compatibility to confirm the absence of antagonistic interactions between them. The presence of an inhibition zone between the streaked isolates indicates antagonistic properties. The results of this study showed that three isolates exhibited synergy, as indicated by the absence of inhibition zones between the isolates (Fig. 1).

Table 4. Biochemical activity of nitrogenase (μM mL^{-1} g^{-1} h^{-1}) and ability nitrogen fixing bacteria to produce the IAA phytohormones (ppm) and organic acid (ppm)

Isolates	Nitrogenase	IAA	Oxalate
Control	0	0	0
N1	0.589 ± 0.20	1.087 ± 0.07	5.981
N3	0.516 ± 0.40	1.146 ± 0.05	5.153
N6	1.377 ± 0.43	1.380 ± 0.08	4.045

Table 5. The synergistic of N1, N3 and N6 isolates using the streak method in NA medium

Isolates	3 DAI	5 DAI	7 DAI
N13	+Syn	+Syn	+Syn
N16	+Syn	+Syn	+Syn
N36	+Syn	+Syn	+Syn
N136	+Syn	+Syn	+Syn

Note: (+syn) grow synergictically.

Plant growth promoting bacteria (PGPB) effect of growth maize seedlings

Consortium of isolate PGPB significantly can increase the growth of maize plants the result highest than control and single isolate. The results of statistical analysis showed a significant effect of PGPB on the growth of maize plants in Fahreus medium as the planting medium consortium treatment was higher than the control (without PGPB) and single PGPB isolates (Table 6).

The plant growth-promoting bacterial strains under investigation significantly enhanced the growth of maize plants (plant height, root length, chlorophyll content, and plant biomass). Inoculation of consortium isolates N136 most significant increase in both plant height and root length, with values of 30.17 cm and 33.33 cm. The result respresent the increment of plant height and root length with the value 68.83% and 115.45%, compared with control (without inoculation isolate). Consortium of PGPB has a capability of nitrogenase activity and represent of the result can increase the clorophyll content index (CCI) and biomass per plant (mg) with values of 4.55 CCI and 0.37 mg. The result respresent the increment of plant height and root length with the value 93.62% and 94.74%, compared with control (without inoculation isolate) compared with control. The three selected isolates has been tested in under challenging conditions with slightly acidic plant medium (pH 6) and low levels of organic carbon and nitrogen. Synergistic of consortium of three selected isolates outperformed under such adverse conditions

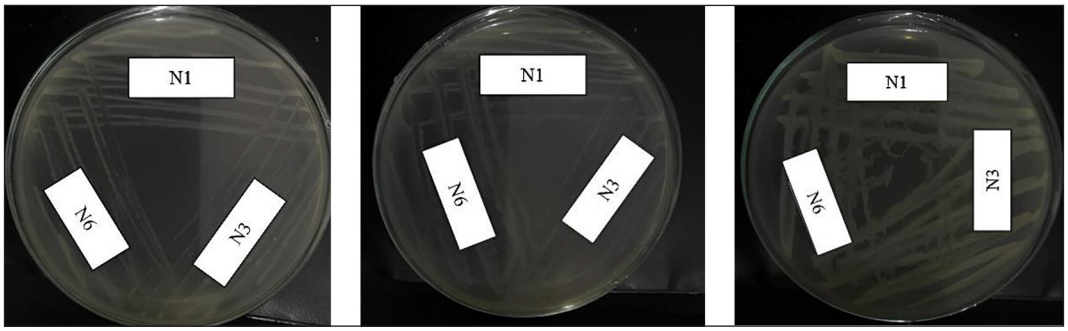

Figure 1. Result of The synergistic of isolates N1, N3 and N6

Table 6. Inoculation isolates PGPB of plant height (cm) and root length (cm) in maize seedlings

Inoculation	Plant height (cm)		Increment plant height (%)	Root Length (cm)		Increment root length (%)
Control	17.87 ±1.60	a	-	15.47 ± 1.94	a	-
N1	23.90 ±0.30	b	34.14	20.53 ± 1.65	b	32.71
N3	23.97 ± 1.90	b	34.14	20.37 ± 0.32	b	31.67
N6	23.50 ± 1.90	b	31.51	18.90 ± 0.85	b	22.17
N13	22.77 ± 1.70	b	27.42	26.83 ± 2.08	c	73.43
N16	24.53 ± 1.20	b	37.27	28.93 ± 0.51	c	87.01
N36	22.70 ± 1.80	b	27.03	26.43 ± 1.45	c	70.85
N136	30.17 ± 0.80	c	68.83	33.33 ± 1.53	d	115.45

Note: The average value followed by the same letter in the same column shows no significant difference at the 5% level according to Duncan's Test.

highlights their adaptability and potential to function effectively in nutrient poor soils. Plant growth promotion bacteria (PGPB) that can increase plant growth and production and can fix N_2, dissolve phosphate, and synthesize the phytohormone IAA (Indole 3-Acetic Acid) (Husna et al., 2020). Individual inoculation of this strain in maize plants showed a significant increase (compared to those not inoculated) in plant height (35–40 %), shoot dry weight (244–289 %), root dry weight (99–137%), and SPAD value (40–47%) (Aviles et al., 2022) (Figure 2). Application PGPB can increase the growth of roots (50–68%), shoots (25–54%), and chlorophyll content. Inoculation 49 Isolates PGPB has resulted increase root length of maize because PGPB can increase the synthesis of phytohormones IAA (Aquino et al., 2019) (Table 7).

The final population of PGPB isolates in the fahreus medium after 21 days after inoculation (DAI) PGPB in bioassay planting media showed that the bacterial population reached 2.20×10^{10} CFU mL^{-1}. The high and low total population of N_2-fixing bacteria is influenced by the organic carbon energy sources available in the rhizosphere environment (Hermiati et al., 2021). The growth of plants is stimulated through the synthesis of compounds that can help in the absorption of nutrients from the environment. Plants play a significant role in influencing the soil environment around their roots, known as the rhizosphere, through various mechanisms. The pH of the soil, its structure, and the availability of oxygen are all affected by the plant's activities. For example, roots can release certain substances that either acidify or alkalinize the surrounding soil, which can influence the types of microorganisms that thrive there. Additionally, plants can alter soil structure by releasing compounds that affect soil aggregation and porosity, impacting water and oxygen flow (Jacoby et al., 2021). Interactions between plants and soil microbes are complex and influence the microbial community's composition and behavior. The changes in the local soil environment, driven

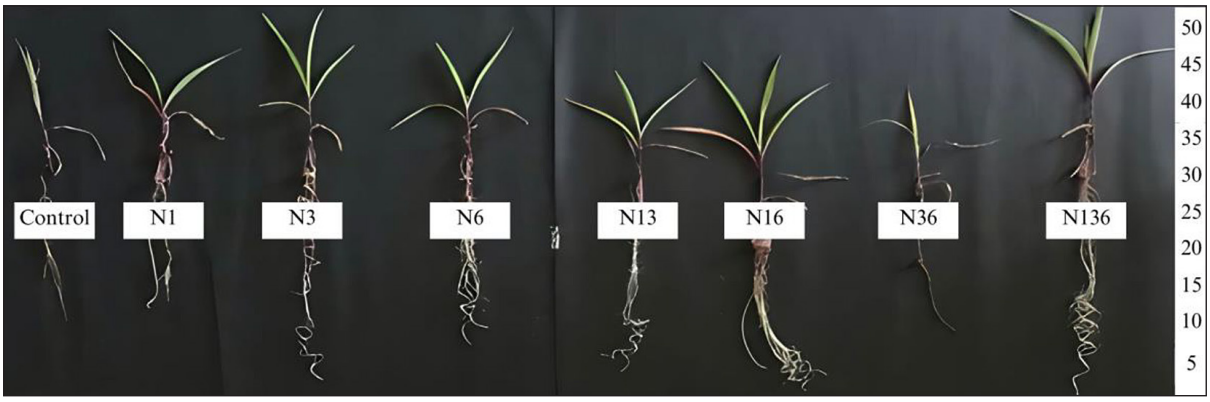

Figure 2. Growth of maize seedling after inoculation of PGPB

Table 7. Inoculation isolates PGPB of chlorophyll content (CCI) and plant biomass (mg) in maize seedlings

Inoculation	CCI		Increment CCI (%)	Plant biomass (mg)	Increment biomass (%)
Control	2.35 ± 0.44	a	-	0.19 ± 0.01	0.00
N1	3.14 ± 0.05	bc	33.62	0.25 ± 0.05	32.71
N3	2.60 ± 0.23	ab	10.64	0.26 ± 0.06	31.67
N6	3.07 ± 0.41	abc	30.64	0.29 ± 0.08	22.17
N13	3.52 ± 0.05	c	49.79	0.32 ± 0.03	73.43
N16	3.17 ± 0.81	abc	34.89	0.34 ± 0.12	87.01
N36	2.81 ± 0.53	abc	19.57	0.35 ± 0.08	70.85
N136	4.55 ± 0.42	d	93.62	0.37 ± 0.04	115.45

Note: the average value followed by the same letter in the same column shows no significant difference at the 5% level according to Duncan's Test.

Table 8. Total population bacteria (TPB) of PGPB in fahreus medium 21 day after inoculation

Treatments	TPB (x 10^{10} CFU mL^{-1})	
Control	1.25 ± 0.22	a
N1	1.57 ± 0.22	a
N3	1.43 ± 0.30	a
N6	1.50 ± 0.14	a
N13	1.69 ± 0.48	a
N16	1.60 ± 0.23	a
N36	1.66 ± 0.48	a
N136	2.20 ± 0.40	b

Note: the average value followed by the same letter in the same column shows no significant difference at the 5% level according to Duncan's Test.

by plant root activities and exudate release, shape the microbial landscape in ways that can support plant growth, influence nutrient cycling, and impact soil health overall (Pantigoso et al., 2022). A comprehensive bioassay testing approach was employed to assess the impact of biofertilizers derived from the three selected isolates PGPB on plant viability. The results of this testing indicated no adverse effects associated with the utilization of these biofertilizers (Table 8).

CONCLUSIONS

The plant growth-promoting bacteria (PGPB) isolates N1, N3, and N6 exhibited synergistic interactions, enhancing their collective efficacy without any antagonistic effects. Applied as a consortium of PGPB to maize plants, this combination significantly improved key growth parameters, including plant height, root length, chlorophyll content, and biomass. The findings highlight the beneficial impact of a well-coordinated PGPB consortium on promoting plant growth. PGPB has a capability of nitrogenase activity, producing IAA and organic acid. The highest of value nitrogenase activity and producing IAA were observed in isolate N6 (1.377 μM mL^{-1} g^{-1} h^{-1} and 1.380 ppm) and the highest of producing organic acid in isolate N1 (5.981 ppm). The population of PGPB in the medium was highest in treatments with the consortium compared to the control (without isolates) and single isolate applications. Inoculation of PGPB consortium has effected on plant growth responses with values of clorophyll content 4.5 CCI, biomass 0.37 mg at 21 days after inoculation (DAI). These values were significantly higher than those observed in control treatments (without PGPB) and single isolate applications. The results indicate that the PGPB consortium can be developed as an effective active ingredient in biofertilizers to sustainably enhance maize growth and productivity and approach to support environmentally friendly agricultural practices. The biofertilizers can be extrapolated to other crops and regions to some extent. The applicability of biofertilizers to other crops and regions is influenced by various factors that determine their effectiveness.

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