Growth Enhancement of *Zea mays* by the Application of Phosphate Solubilizing Bacteria

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ABSTRACT

The efficiency of applied phosphorus rarely exceeds 30% due to fixation in soil; it is also lost as a result of run-off and leaching, leaving as little as 10–20% available for plant utilization. The study was aimed at isolating and characterizing phosphate-solubilizing bacteria and their application as growth-promoting agent for maize cultivation. Composite soil samples were collected from three (3) different soils around the plant roots while brackish water was collected from the New Calabar River. Maize (*Zea mays*) seeds were purchased from Mile 3 Market, Port Harcourt, for the analysis. The seeds were cultured according to the standard microbiological procedures using Pikovskaya medium (PVK) and Nutrient agar (NA). Isolates were identified and screened for their phosphate-solubilizing potential. Bacteria with higher solubilizing potential were applied to grow maize seedlings. The results showed that soil samples contained high populations of heterotrophic and phosphate-solubilizing bacteria (PSB), with PSB counts ranging from $1.85×10^4±0.36$ to $1.57×10^5±0.55$CFU/g, and heterotrophic counts ranged from $3.49×10^4±0.43$ to $4.80×10^6±0.14$CFU/g, while brackish water had the lowest bacterial population. The phosphate solubilizing index (PSI) ranged from $2.0±0.03$ to $3.0±0.04$, with *Pseudomonas* spp. showing the highest solubilizing ability and *Staphylococcus* spp. the lowest. Maize plants inoculated with a consortium of *Enterobacter*, *Bacillus*, and *Pseudomonas* recorded the best growth performance with highest plant height of 178.5cm, leaf number of 12, leaf length of 94cm, and earliest fruiting at week 7. Among single-strain treatments, *Bacillus* promoted the highest plant height, leaf number, and fruit production at 8 weeks, while *Pseudomonas* showed the greatest leaf length. *Enterobacter* performed moderately, whereas NPK and the control recorded the lowest growth metrics across all parameters following its normal growth duration. Notably, fruiting was first observed in the PSB consortium treatment in week 7, while plants treated with single PSB cultures of *Pseudomonas*, *Bacillus*, or *Enterobacter* began fruiting at week 8. The phosphate-solubilizing bacteria (PSB) consortium demonstrated superior growth-promoting effects on maize compared to single isolates, NPK, and the control; by not only enhancing plant height, leaf number, and leaf length but also accelerating fruit production, indicating its potential as an efficient biofertilizer for improved maize productivity.

Keywords: Maize (*Zea mays*), Composite Soil, Phosphate Solubilizing Bacteria, Consortium, Phosphate Efficiency.

Introduction

The application of fertilizer both organic and synthetic is aimed at improving the growth of plants in a bid to increase yield. Apart from nitrogen (N) and potassium (K), phosphorus (P) is an important macronutrient to sustain crops yield for the production of both food and feed (Scholz and Geissler, 2018; Sarkar *et al*., 2018).

Phosphorus is an important macronutrient which is directly involved in nucleic acids, cells division and growth of new tissues, which also control or regulate protein synthesis and energy transfer (Elhaissoufi *et al*., 2022). This nutrient is required in different cellular processes such as photosynthesis, carbohydrate metabolism, energy production, redox-homeostasis, and signaling.

Phosphorus plays a crucial role in root development, root characteristic anatomy modifications and root hair density with a crucial contribution in the increase of crop yield.

The absence of available phosphorus in the right quantity by the soil or fertilizer can result in the limitation of normal plant growth (Elhaissoufi *et al.*, 2022). Phosphorus is the second most important nutrient for plants, after nitrogen. It exists in soil as mineral salts or incorporated into organic compounds. Despite these phosphorus compounds being abundant in agricultural soils, the majority of them occurs in an insoluble form (Oteino *et al.*, 2013).

Following its arrival in West Africa in the 15th century, maize became an invaluable crop fitting into the existing diverse farming systems because of its broad adaptation to varying growing conditions, ease of processing and resistance to pre-harvest bird damage that plagued sorghum, millet, and rice (Fakorede *et al*., 2022). As a quick maturing crop, it has become a critical source of food for rural families when food reserves are depleted before the root crops, sorghum, millet, and other native crops are harvested (Wossen *et al.,* 2023).

Plants require approximately 30µmol l-1 of phosphorus for maximum productivity, but only about 1µmol l^{-1} is available in many soil environments. Therefore, the unavailability of phosphorus in many soils has been recognized as a major growth limiting factor in agricultural and horticultural systems (Oteino *et al*., 2015). As a result of the crucial role played by phosphate solubilizing bacteria in plant growth and yield, there is need to understand the impact of the effect on the growth of plants (as biofertilizer) on monocotyledon crop (maize) which is one of the most eaten staple food in Nigeria.

Materials and Methods

Sample Collection

Three soil samples were collected from plant roots in three different farms at Rivers State University Demonstration farm, while a sample of brackish water was collected from the New Calabar River. The collected samples were transported in sterile zip-locked bags and sterile bottles to the Rivers State University, Microbiology laboratory for immediate analysis.
The seeds of maize (*Zea mays*) were purchased from the Mile 3 Market, Port Harcourt, for the analysis.

Isolation of Test Organisms

Pikovskaya's agar (PVK) medium containing Glucose, 10g, $Ca_3(PO_4)_2$, 5g; $(NH4)_2SO_4$, 0.5g, $MgSO_4.7H_20$, 0.1g, KCl, 0.2g, Yeast extract, 0.5g, $MnSO_4.H_2O$, 0.002g, $FeSO_4.7H_2O$, 0.002g and Distilled water, 1Litre was used in the isolation of phosphate solubilizing bacteria. Nutrient agar was used in the determination of total heterotrophic bacterial counts (THBC) of the samples. The samples each of 10g (for the soil sample) or 10ml (for the water sample), were resuscitated in sterile normal saline (of 90ml) and 10-fold serial dilution was carried out. An aliquot (0.1ml) of the diluted samples were inoculated on the prepared media plate and spread with the aid of a sterile hockey stick using spread plate method. The inoculated plates were incubated for 24-48 hours at 37°C. After incubation of the plates, the Total count of the bacteria was determined by counting the colonies on the plates and the CFU/g (colony forming unit per gram) were calculated. The various isolates on the culture plate were purified by streaking the bacterial isolates on a freshly prepared nutrient agar plates based on their various cultural morphological characteristics and incubated at 37°C for 24 hours to have a pure culture of the isolates (Nrior *et al.*, 2022).

Biochemical Tests

Biochemical test carried out in this research were; Methyl Red Voge-Proskauers (MRVP) test, sugar fermentation tests, catalase, indole, production test, test for hydrogen sulphide and gas production, citrate utilization test, sugar fermentation (Talaiekhozani *et al.,* 2015).

Screening for the Phosphate Solubilizing Potential

Adopting the method of Sharon *et al.* (2016) the isolates from initial Pikovskaya (PVK) media plates were inoculated on a freshly prepared PVK agar medium with the use of an inoculating needle and incubated for 4-7days for better analysis of zone of clearance. The bacteria with phosphate solubilizing potential produced a clear zone of inhibition which were measured and phosphate solubilizing index was calculated using the formula;
Phosphate solubilizing index (PSI) =

$$\frac{colony\ diameter\ +\ clearance\ zone}{Colony\ diameter}$$

Application of Phosphate Solubilizing Bacteria as Growth Enhancer for Maize (Zea mays)

An experiment to study the phosphorus mobilization to plants by selected phosphate solubilizing bacteria was conducted in bags (15kg each) on the field using Maize (*Zea mays*). Horticultural soil was used as the growth substrate in this experiment. The maize seeds were surface sterilized using 70% ethanol and were washed twice with sterile distilled water. The seeds were soaked in bacteria (with high solubilizing index) that is the *Enterobacter sp, Bacillus sp, Pseudomonas sp,* and the consortium in an overnight culture suspension for 48 hours at 28 ±2 ℃ and were washed once with sterile distilled water (Wang *et al.*, 2022). The seeds were planted in a nursery bed for growth.

The seedlings which grew (after 7 days) were planted in soil bag for each treatment. Four seeds of *Zea mays* soaked with appropriate bacterial inoculum were sown in each bag. The experimental treatments consisted of triplicate bags containing;

(a) inoculated seed and bacteria 1; (b) inoculated seed with bacteria 2; (c) inoculated seeds with bacteria 3; (d) inoculated seed with the mixture of the three bacteria (e) un-inoculated seeds as a negative control; and (f) un-inoculated seeds sown in soil fertilized with NPK (15:15:15) fertilizer (presented in Table 1). The maize seedlings were transplanted into the bags from the nursery bed after 7 days of germination, then after a week of planting in bags, (5g) of NPK 15:15:15 fertilizer was applied around each plant 5-7cm away from the stem in a ring form and cover lightly with soil and water was applied immediately, and repeated at two weeks interval (Wang *et al.*, 2022).

Then two 2ml of PSB was diluted with 8mls of distilled water and applied in a ring form as well around the plant 5-7cm away from the stem. All plants were cultivated in the bag and were watered twice per week. Growth parameters such as root and leaf length, number of leaves, number of fruits were determined (Wang *et al.*, 2022).

Table 1: Plant Treatment Setups

Plant setups	Planted Maize	*Enterobacter*	*Bacillus*	*Pseudomonas*	NPK
Maize Planted in 15kg of soil only (Control)	+	-	-	-	-
Maize Planted in 15kg of soil + *Enterobacter*	+	2ml	-	-	-
Maize Planted in 15kg of soil + *Bacillus* sp	+	-	2ml	-	-
Maize Planted in 15kg of soil +*Pseudomonas*	+	-	-	2ml	-
Maize Planted in 15kg of soil +*Enterobacter*+*Bacillus* + *Pseudomonas* sp	+	0.67ml	0.67ml	0.67ml	-
Maize Planted in 15kg of soil +NPK	+	-	-	-	5g

Key: NPK = Nitrogen, Phosphorus and Potassium

Results

The bacterial population (counts) of the samples are presented in Table 2. The counts of phosphate-solubilizing bacteria (PSB) of the soil sample ranged from $1.85×10^4±0.36$ CFU/ml to $1.57×10^5±0.55$ CFU/g, with the least count obtained in the brackish water sample and the highest counts obtained in the soil sample (sample C). The total heterotrophic counts ranged from $3.49 ×10^4 ±0.43$ CFU/ml to $4.80 ×10^6 ±0.14$ CFU/g with the highest counts recorded in the soil sample (sample C) while the least counts of heterotrophic bacteria was obtained in the brackish water sample.

Table 2: Bacterial Population (CFU) of the Soils and Brackish Water Samples

Samples	Phosphate Solubilizing bacteria Count	Total Heterotrophic Bacterial Count
Soil A	$6.15 ×10^4±1.202$ CFU/g	$2.72 ×10^5±2.08$
Soil B	$1.20 ×10^5±0.77$ CFU/g	$5.10 ×10^5±0.70$
Soil C	$1.57 ×10^5±0.55$ CFU/g	$4.80×10^6±0.14$
Brackish water	$1.85×10^4 ±0.36$ CFU/ml	$3.49 ×10^4 ±0.43$

The different phosphate solubilizing bacteria identified were; *Bacillus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Enterobacter* sp, *Klebsiella* sp, *Pseudomonas* sp, *Citrobacter* sp, and *Serratia* sp were identified with the help of Bergey's Manual of Systemic Bacteriology (Whitman *et al.,* 2012).

The phosphate solubilizing bacterial genera isolated and their percentage abundance were: *Bacillus* (27.7%),*Pseudomonas* (16.7%), *Micrococcus* (13.9%), *Enterobacter* (11%), *Staphylococcus* (8.3%), *Citrobacter* (8.3%), *Proteus* sp (6%), *Serratia* (5.5%), and *Klebsiella* (2.9%). The bacterial genus with the highest percentage abundance was *Bacillus* sp followed by *Pseudomonas* and *Enterobacter* while the bacterial genus with the least percentage abundance was *Klebsiella*. The phosphate solubilizing potential of the bacterial isolates defined by the phosphate solubilizing index. The phosphate solubilizing index ranged from 2.0±0.03 to 3.0±0.04 with *Pseudomonas* spp. recording the highest PSI of 3.0±0.04 and *Staphylococcus* spp. with the least PSI of 2.0±0.03.

The pictorial effect of the isolates of phosphate solubilizing bacteria on the growth and length of leaves of maize (*Zea mays*) plant at 7 weeks is presented in Plate 1.

The effect of the isolates of phosphate solubilizing bacteria on the growth (height) of maize plant is shown in Figure 1. Among the inoculated treatments, the treatments with consortium of *Enterobacter +Bacillus+Pseudomonas* strain showed maximum plant height (178.5±7.77 cm) of maize at 7 Week (49 days) followed by the amendment treatment with *Bacillus* sp (138.5±31.81 cm) followed by the plant with the treatment with *Pseudomonas* (130.0±42.42 cm) followed by the treatment of the maize plant with *Enterobacter* (115.5±36.06 cm) followed by treatment with NPK (72.5±3.5 cm) and the control setup (70.00±0.00 cm).

The effect of the isolates of phosphate solubilizing bacteria on the number of leaves of maize *Zea mays* plant is shown in Figure 2. Among the inoculated treatments, the treatments with consortium of *Enterobacter +Bacillus+Pseudomonas* strain showed highest number of leaves (12.0±1.41 cm) of maize at 7 week(49 days) followed by the amendment with treatment with *Bacillus* sp (11.5±0.70 cm).

Plate 1: Planted maize at week 7

Key: Line 1 (from the left) is the Planted Maize amended with NPK; the last two lines to the right are the Planted Maize amended with phosphate solubilizing bacteria (PSB).
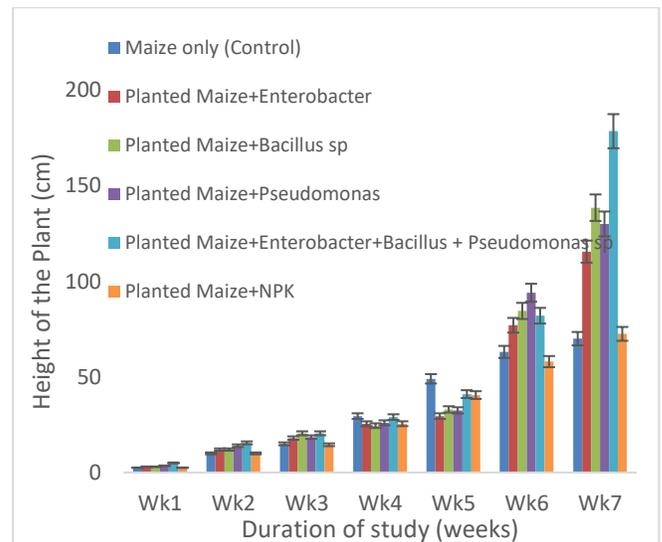

Figure 1: Height of the plants in the setups during the study period

The amendment treatment with *Bacillus* sp (11.5±0.70 cm) was followed by the plant with the treatment with *Pseudomonas* (11.0±0.00 cm) followed by the treatment of the maize plant with *Enterobacter* (8.5±0.70 cm) followed by treatment with NPK and control (7.0±00 cm).
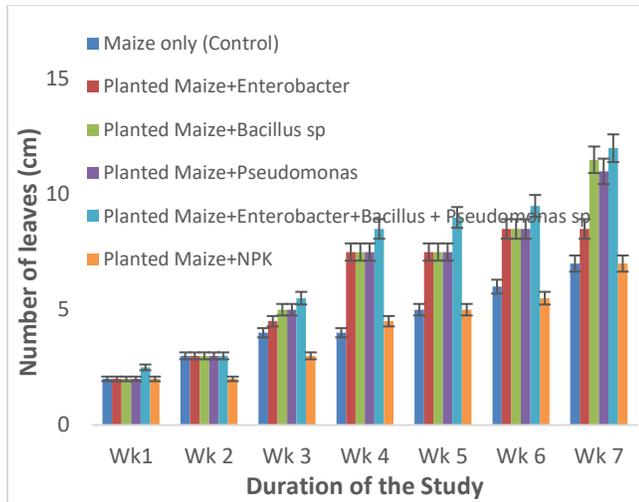
Figure 2: Number of Leaves of maize (*Zea mays*) during the study period

Figure 3: Length of Leaves of maize (*Zea mays*) during the study period

The effect of the isolates of phosphate solubilizing bacteria on the growth (length of leaves) of maize (*Zea mays*) plant is shown in Figure 3. Among the inoculated treatments, the treatments with consortium of *Enterobacter*+*Bacillus*+*Pseudomonas* strain showed highest length of leaves (94.0±2.82 cm) of maize at 7 week (49 days) followed by the amendment treatment with *Pseudomonas* sp (88.5±0.70 cm) followed by the plant with the treatment with *Bacillus* (80.50±0.70 cm) followed by the treatment of the maize plant with *Enterobacter* (77.5±4.94 cm) followed by treatment with NPK (55.5±2.12 cm) and the control setup (52.50±3.53 cm).

The effect of phosphate solubilizing bacteria on the production of maize fruit is shown in Table 3. The treatment setup with the phosphate solubilizing bacteria consortium fruited at week 7.
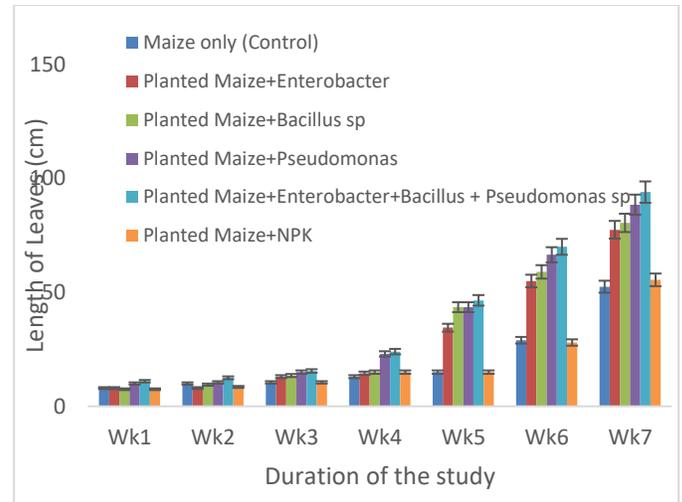
While other treatment setups with the single PSB isolate of either *Pseudomonas*, *Bacillus* or *Enterobacter* showed the production of fruit at week 8. Additionally, at week 8, no fruit production was observed on the plants in the control setups and those of amendment with NPK.

At week 9 and 10, two and three fruits were recorded in the treatment of bacterial consortium while the treatment setup with *Bacillus* and the treatment with *Pseudomonas* recorded two fruits in the 10th week whereas the treatment setups with NPK recorded and control setup recorded one fruit respectively. At week 10, the highest size (15cm) of the fruit produced (measured by their circumference) was observed in the plants in the consortium treatment while the least size (6cm) was recorded in the control samples.

Table 3: Production of Fruits by phosphate solubilizing bacteria during the study period

Plant setups	Number of Fruits Produced				Circumferences of fruit			
	Wk 7	Wk 8	Wk 9	Wk 10	Wk 7	Wk 8	Wk 9	Wk 10
Maize only (Control)	-	-	-	+	-	-	-	6.25cm±0.35
Planted Maize+*Enterobacter*	-	+	+	+	-	-	7cm ±0.00	10.5cm±0.21
Planted Maize+*Bacillus* sp	-	+	+	+ +	-	7.2cm±0.14	10.0cm ±0.00	13.0cm±0.00
Planted Maize+*Pseudomonas*	-	+	+	+ +	-	-	5.2cm ±0.28	10cm±0.00
Planted Maize+*Enterobacter*+ *Bacillus* + *Pseudomonas* sp	+	+	++	+ + +	5cm±0.0	7.2cm±0.28	11.1cm±0.14	15.3cm±0.42
Planted Maize+NPK	-	-	-	+	-	-	5.15cm±0.212	10.15cm±0.21

Key: + = One fruit out of the three bags, ++ = Two fruits out of the three bags**,** +++ = Three fruits out of three bags

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Discussion

Bacteriological analyses revealed that phosphate-solubilizing bacteria (PSB) populations were substantially higher in soils than in the brackish water sample. The total heterotrophic bacterial count followed a similar trend, with Soil C showing particularly high abundance in the soil samples compared to the aquatic sample suggesting high bacterial activity, which could be attributed to nutrient availability and organic matter content (Elhaissoufi *et al.*, 2021). This finding is in consonance with the observation in the study of Douglas *et al*. (2024) and Asuming-Brempong and Afere, (2014) which reported that higher counts of PSB and THB were recorded in soil sample compared to the aquatic samples. The population of PSB in the soil depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities. Larger populations of PSB are found in agricultural and range land soils (Fenta, 2017).

A diverse range of bacterial genera were identified, including *Bacillus, Pseudomonas, Enterobacter, Micrococcus, Staphylococcus, Citrobacter, Serratia, Proteus* and *Klebsiella*. These genera are similar to those reported in other studies (Douglas *et al*., 2022; Elhaissoufi *et al.,* 2021; de Amaral *et al.,* 2020). According to Nadieline *et al*. (2019), abundance and diversity of microorganisms in the rhizosphere are likely to be related to plant species on the soil due to differences in root exudation and rhizodeposition.

Bacillus sp was the most abundant bacteria isolated, followed by *Pseudomonas* sp. *Pseudomonas* spp., although less abundant (16.7%), recorded the highest phosphate-solubilizing index (PSI = 3.0 ± 0.04), followed by *Bacillus* sp (2.4.00 ± 0.03) underscoring their potential as efficient biofertilizers. This finding is in line with the report of Sharma *et al*. (2013) and Douglas *et al.,* (2024), which reported similar organisms (*Pseudomonas* sp) with the highest potential for phosphate solubilization.

This taxonomic profile aligns with reports that *Bacillus* and *Pseudomonas* are dominant PSM genera in diverse ecosystems due to their metabolic versatility, resilience, and ability to produce organic acids that solubilize insoluble phosphates (Igoni *et al.*, 2023).

Bacillus spp. due to its capacity to form spores and has been used to produce biofertilizers since it can tolerate harsh environmental conditions and remain in the soil environment for a prolonged period (Douglas *et al.,*2018). In another study by Sarker *et al.,* (2014) to isolate phosphate-solubilizing bacteria that increase growth and improve nutrient uptake by wheat, the genus, *Pseudomonas* was also isolated and identified to have better phosphate-solubilizing potential, as a result, increasing the growth of wheat. The greater potential of *Pseudomonas* sp to solubilize phosphate may be due to its capacity to produce more organic acid during microbial metabolism (Demissie *et al.,* 2013). Previous studies have reported that strains of *Bacillus licheniformis* and *Bacillus amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids (Kumar *et al.*, 2018). Additionally, studies have recorded that Gram-negative bacteria are more effective at dissolving mineral phosphates than Gram-positive bacteria due to the release of diverse organic acids into the surrounding soil (Kumar *et al.*, 2018).

Citrobacter are found in water, soil and human intestines and it utilize citrate as sole carbon source (Sadiq *et al*., 2013). The genus *Pseudomonas* is one of the diverse groups of bacteria found in water, soil, plants and animal tissues. It belongs to the family Pseudomonadaceae and can tolerate a range of physical environment. Isolates with great gene match to *Pseudomonas putida* from this study had the ability to solubilize phosphate as was also demonstrated by Leontidou *et al*, (2020) in their study. *Bacillus* is one of the most studied and diverse bacteria of the family Bacillaceae. Some members from this genus have been proven to be among the best phosphate solubilizers (Kirui *et al.,* 2021).

In the application study of the isolated phosphate-solubilizing bacteria in the growth of maize, the treatments with consortium of *Enterobacter* +*Bacillus*+*Pseudomonas* strain resulted in increased growth in the height of the maize plants, number of leaves and largest size of leaves at 7 week (49 days) followed by the amendment treatment with *Bacillus* sp followed by the plant with the treatmented with *Pseudomonas,* followed by the treatment of the maize plant with *Enterobacter* followed by treatment with NPK (72.5±3.5 cm) and the control setup.

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From the results of this study, *Bacillus* and *Pseudomonas* produced more evident of increased growth in the measured parameters when applied alone and their combination with *Enterobacter* produced better results and this suggest synergism of the phosphate-solubilizing bacteria in the growth of Maize. Furthermore, at week 8, the treatment setups with the consortium started producing fruit while the other treatment setups amended with the single organisms started producing at week 8. This is in agreement with the finding of other studies. According to the study by Fenta (2017), the stimulation of crop growth by PSB (*Pseudomonas putida* and *P. flourescen*) resulted in the increased root and shoot elongation in canola, lettuce, tomato, as well as crop yields in potato, rice, tomato, lettuces, apple, citrus, beans, ornamental plants and wheat (Fenta, 2017). The effect in the length of leaves is similar to the report by Mehrvarz *et al.,* (2008) which observed increase leave chlorophyll content as a result of the biofertilization by PSB (Mehrvarz *et al.*, 2008).

Under good conditions and with an early-maturing variety, maize gets to maturity and start producing fruits (milk stage) within about 2.5 to 3 months, and reach full dry-grain maturity in roughly 3 to 4.5 months (Wossen *et al.,* 2023). However, in the present study, the production of fruit with the treatment setup of consortium of PSB showed earlier yield of the maize fruits including the maize setup with NPK fertilizer which can be attributed to the positive effect of the phosphate solubilizer (Fenta, 2017). The bioformulation by biofertilizer such as phosphate solubilizing bacteria as a process, is a crucial multistep consisting of providing a safe environment that protects microbial cells once they are introduced through a suitable carrier into the soil (Elhaissoufi *et al.*, 2022).

Conclusion
The study identified several phosphate-solubilizing bacteria, with *Bacillus* and *Pseudomonas* species being the most prevalent and effective. These bacteria enhanced maize growth and yield. Their combined use (consortium) showed synergistic effects, outperforming conventional NPK fertilizer. The findings highlight the potential of phosphate-solubilizing bacteria as sustainable biofertilizers that can reduce reliance on chemical fertilizers and promote environmentally friendly agriculture.

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