SCREENING OF PLANT GROWTH PROMOTING BACTERIA (PGPB) FROM THE SOIL AND THEIR EFFECTS ON THE GROWTH OF SELECTED PLANTS*

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Abstract

Microorganisms in soil play a crucial role in crop production and sustainable development of agriculture. This study was intended to isolate the PGPB strains from different soil sample. The experiment was performed at the Molecular Research Laboratory, Department of Botany, Center for Research and Innovation, University of Yangon, in 2024. In this study, five bacterial strains have been isolated and given named as SP1 to SP5. In morphological characterization, all strains showed the circular shape, entire margin, raised elevation, smooth texture and transparent opacity except the opaque opacity in the strain SP1. Microscopical observations revealed that strains SP1, SP2, SP4 and S5 are bacilli shape, Gram-positive bacteria and only SP3 are Gram-negative bacteria with the form of bacilli. The different biochemical tests and nitrogen fixation test of each isolated strains were performed. The DNA extraction and purification from each strain were conducted and all extracted bacterial DNA produce relatively high purity of DNA, ranging between 1.8 and 2.0 in A_{260}/A_{280} ratio with less impurities in A_{260}/A_{230} ratio. Among five strains, strain SP2 and SP5 indicated the best DNA purity result. Moreover, the improvement of plant growth promotion performance indicated the positive effects on the selected plants at 15 days post inoculation, especially strains SP2 and SP5 showed the maximum results on plant growth parameters.

Keywords: soil bacteria, plant growth promoting bacteria (PGPB), DNA extraction, growth parameters

Introduction

Sustainable agricultural development is particularly important for world's population estimated to reach 9.1 billion by the year 2050 due to the necessity of improvement and enhancement of productivity of agricultural products (FAO, 2009). To meet the improvement of crop production, modern agriculture production strongly relies on synthetic fertilizers and pesticides to achieve high productivity levels. However, it can impact the decreasing organic matter content, soil degradation, pollution in groundwater system, and causing severe diseases (Adesemoye *et al*., 2009, Bourguet and Guillemaud, 2016). Therefore, it is important to find eco-friendly and sustainable alternative from natural resources and microbiological approaches have become more popular for crop improvement. Nowadays, plant growth promoting bacteria (PGPB) are considered as environmental friendly and easily biodegradable for the enhancement of plant productivity (Jat Baloch *et al*., 2023).

PGPB belongs to the free-living group of soil bacteria which improve both plant growth and crop yield when they are applied in different ways (Kumar *et al*., 2014). PGPBs, initially described by Kloepper and Schroth in 1978, are mainly defined as a heterogeneous group of bacteria that promote the growth of plants by affecting the soil environment through direct or indirect mechanisms (Goswami *et al*., 2013 and Wang *et al*., 2014). PGPB belong to a vast variety of genera such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Di Benedetto *et al*., 2017) which are applied to the production of various crops such as cereals and

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vegetables (Basu *et al*., 2021). PGPB stimulate plant growth by various mechanisms including improvement of plant nutrition, unique enzymes secretion, phytohormones regulation, and suppression of pathogenic organisms. The exploitation of PGPB could reduce the application of synthetic fertilizers and help maintain soil health (Benizri, *et al*., 2001).

Many recent studies have expanded the term PGPR to plant growth promoting bacteria (PGPB) by including various bacterial strains from a diverse range of sources, rather than rhizosphere (Goswami *et al*., 2013). Among diverse PGPB groups, *Bacillus* spp. is the safe microorganisms that hold remarkable abilities for synthesizing a vast array of beneficial substances and it possess the potential plant growth promoting traits (Stein, 2005). In addition, the process of isolating bacterial DNA can be crucial step in identifying and studying beneficial bacteria (PGPB) when applied to plants, which can significantly enhance their plant growth by mechanisms like nutrients solubilization, hormone production and pathogen suppression. Purified DNA from soil bacteria can be used to study the role of soil microorganisms in plant growth (Parthasarathy, *et al*., 2025).

Therefore, the isolation of indigenous PGPB strains with plant growth promoting potential is necessary for sustainable agricultural development. For this reason, the present study was proposed to isolate and characterize the PGPB strains from different soil samples, to conduct the morphological, microscopical and biochemical characterization, to extract and purify the genomic DNA from bacterial isolates, and to evaluate the effect of plant growth promotion on selected plants.

Materials and Methods

1. Sample Collection

The soil samples were collected from different soil in San Phu village, Meneitaung area, Hopong Township, Southern Shan State at the GPS location 20°39′51″N 97°18′3″E, 20°40′52″N 97°18′8″E and 20°40′42″N 97°17′57″E. The collected samples were placed in sealed plastic bags.

Figure. 1. Loacation map of Sample Collection

2. Isolation of Bacteria from Soil Samples

The soil microorganisms were isolated by serial dilution technique (Phay and Ando 2019). In this method, 2 g of soil samples was suspended in 18 ml of distilled water. Then, the suspension was shake for 30 minutes and transferred 2 mL into new tube containing 18 ml of distilled water. Mixed them very well and transferred 1 mL into new tube with 9 mL DW (1/1000). From the total 10mL tube, transferred 2 mL to new tube containing 8 mL of DW

(1/5000) and 1 mL to 9 mL tube (1/10000). The dilution series were prepared between 10^{-1} to 10^{-5}. These dilutions were spread on nutrient agar medium (NAM) and incubated for 24 hours at room temperature. After incubation, pure culture maintained at 4 °C for further studies.

3. Morphological and Microscopical Characterization

The morphological characteristics of isolates such as colony color, shape, elevation, margin, optical density and size were determined by observing the colonies on the plates. Microscopical characterization was examined by Gram staining (Bergey, *et al*., 1994).

4. Biochemical Characterization

Biochemical tests such as (1) Catalase test, (2) Citrate utilization test, (3) Gelatinase hydrolysis test, (4) Indole test, (5) Methyl red (MR) test, (6) Motility test, (7) Vogas Proskauer (VP) test, (8) Triple sugar iron (TSI) test, and (9) Urease test, were performed (MacFaddin 2000). Similarly, the estimation of N-fixation was checked using N-free Jensen's agar medium (Kayasth *et al*., 2014).

5. Extraction and Purification of Genomic DNA

The genomic DNA extraction from bacterial isolates was conducted by using the Wizard® Genomic DNA Purification Kit (Promega Corp. 2005). First, 1ml of overnight culture was centrifuged for 3 minutes at 14,000 rpm to get the pellet cells and discard the supernatant. For Gram positive bacteria, suspend cells in 480µl 50mM EDTA and add 120µl of lysozyme. Then, incubate at 37°C for 1 hour and centrifuge at 14,000 rpm. After, remove the supernatant and add 600µl of Nuclei Lysis Solution. Again, incubate for 5 minutes at 80°C, then cool to room temperature and add 3µl of RNase Solution. The cell incubates again at 37°C for 1 hour, then cool to room temperature. For Protein Precipitation, 200µl of Protein Precipitation Solution add to the tube and incubate on ice for 5 minutes. Then, centrifuged at for 3 minutes and the supernatant transferred to a clean new tube containing 600µl of isopropanol. Then, centrifuged again and decant the supernatant. The 600µl of 70% ethanol added to the tube and centrifuged again. The DNA pellet was air-dried for 10-15 minutes and rehydrated with 100µl of Rehydration Solution. Then, incubated for 1 hour at 65°C or overnight at 4°C for further studies. After the DNA isolation studies, checking the DNA concentration and purity of bacterial strains were performed by using NanoDrop NanoPhotometer® N60 based on the absorbance ratio at 260 and 280 nm and other contamination at 260 and 230 nm (Desjardin & Conklin, 2010). To check the DNA quality, 1 µL sample was pipetted onto the lower pedestal of NanoDrop apparatus. The ratio of A_{260}/A_{280} values between 1.7 and 2.0 indicate DNA samples with good quality. The A_{260}/A_{230} ratio should be greater than 1.5, ideally close to 1.8 (Brescia, 2012 and Vesty *et al*., 2017).

6. Evaluating the Effect of Bacterial isolates on Plant Growth Promotion

The plant inoculation experiment, including all the process (bacterial inoculum and seed preparation, and experimental design for plant assay) were performed. In the current study, black gram, red flat bean and corn were selected for plant inoculation test. Firstly, fresh bacterial culture was prepared 1 day before seeds treatment. The isolates were grown in falcon tube containing 10 mL of Nutrient broth (NB) and incubated in a shaking condition at 150 rpm for 48 hours at room temperature. Then, seeds were washed thoroughly with distilled water for 10 times. Again, the seeds were sterilized with 70% (v/v) ethanol for 3 min and properly washed again with distilled water. Then, placed on the sterilized paper for 2-3 days and germination

percentage were analyzed. The soil was sterilized by autoclaving at 121°C for 15 minutes. Then, good, germinated seedlings were selected and transferred to the plastic cup (top) containing autoclaved soil and each cup containing 1 seed, and 1 ml of bacterial inoculum ($1x10^9$ CFU/ mL) was added to each cavity of seedling. The cup (bottom) was filled with sterilized distilled water. Non-inoculated seedlings were used as a control. These cups were kept in a place full of sun light for proper growth. Plant weight, plant height, root length and root weight of selected plants were evaluated after 15 days of post-inoculation.

Results

1. Morphological and Microscopical characterization of isolated bacteria

The five bacterial strains have been isolated from different soil samples. Among them, strains (SP1 to SP5) showed the circular shape, entire margin, raised elevation, smooth texture, and transparent opacity except with the strain SP1 showed opaque opacity in morphological characterization. Microscopical observations showed all bacterial strains are bacilli shape, Gram-positive bacteria and SP3 was Gram-negative bacteria with bacilli form.

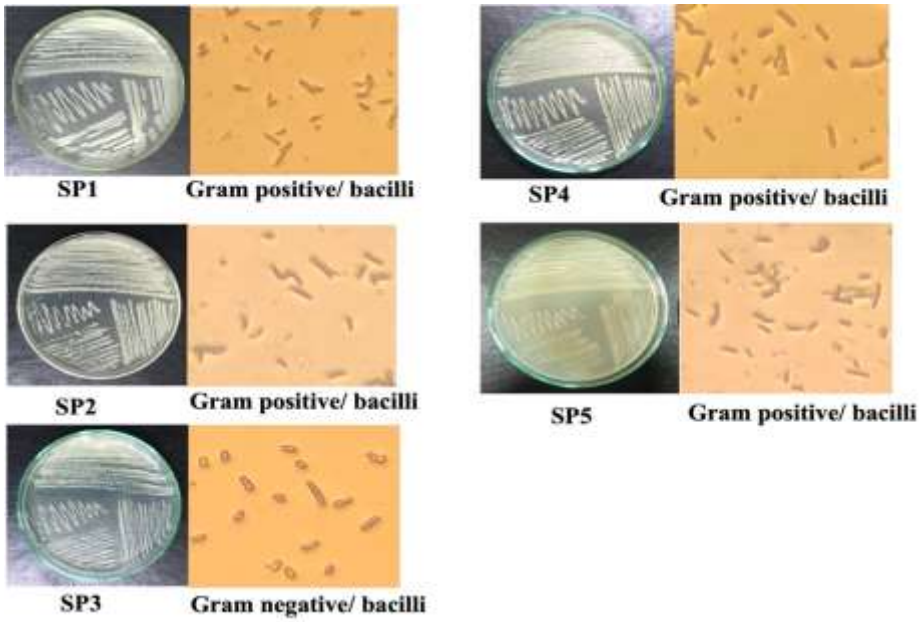

Figure.2. Morphological and Microscopical Characters of isolated strains (X 400)

Table. 1. Morphological and Microscopical Characterization of Five Bacterial Strains

No	Shape	Margin	Elevation	Texture	Opacity	Colony Color		Gram Staining
						Surface color	Reverse color	
SP1	circular	entire	raised	smooth	opaque	white	white	+
SP2	circular	entire	raised	smooth	transparent	white	white	+
SP3	circular	entire	raised	smooth	transparent	white	cream	-
S4P	circular	entire	raised	smooth	transparent	yellow	yellow	+
SP5	circular	entire	raised	smooth	transparent	yellowish cream	yellowish cream	+

2. Biochemical Characterization

Table. 2. Biochemical Characterization of Five Bacterial Strains

No.	Catalase test	Citrate test	Gelatin hydrolysis test	Indole test	(MR) test	Motility test	(TSI) test	Urea Test	(VP) test	N-fixation
SP1	+	-	+	-	-	+	A/A	+	-	+
SP2	+	+	+	-	-	+	K/A (H_2S)	+	-	+
SP3	+	-	-	-	-	+	A/A	-	-	+
SP4	+	-	-	-	+	+	A/A	-	-	+
SP5	+	+	-	-	+	+	K/K (H_2S)	-	-	+

+ = positive, - = negative, K/A= al/ac - dextrose only fermented, A/A= ac/ac - dextrose, lactose, sucrose fermented, K/K= al/al no sugar fermented, Black = H_2S production

Figure. 3. Biochemical Characters of isolated bacterial strains

3. Extraction and Purification of Genomic DNA

The concentration and purity of bacterial DNA were analysed by using NanoDrop spectrophotometer which measure A_{260}/A_{280} and A_{260}/A_{230} ratio. The DNA ratio between A_{260}/A_{280} indicated 1.8 to 2.0 which means all strains indicated the best DNA quality. The strain SP5 showed the best DNA concentration. In A_{260}/A_{230} ratio, the value showed less than 2.0 indicates the presence of contamination. However, strains SP2 and SP5 showed the less contaminations.

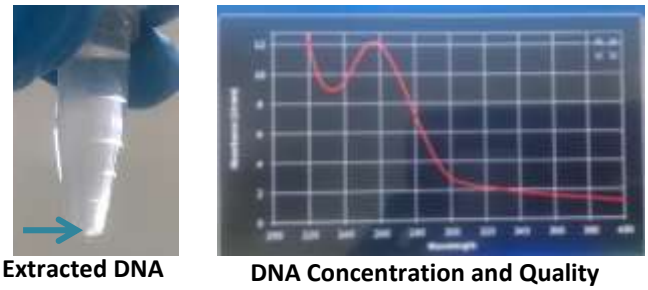

Figure. 4. Extracted DNA and DNA Concentration and Quality Using NanoDrop Spectrophotometer

Table. 3. Genomic DNA Concentration and Quality Using NanoDrop Spectrophotometer

Strains	DNA Concentration (ng/µL)	Indication Ratio for DNA purity (A_{260}/A_{280} = ~1.8 -2.0)	Indication Ratio for Impurities Contamination (A_{260}/A_{230} = 2.0-2.2)
SP1	125.65	1.878	1.166
SP 2	69.250	2.031	1.345
SP 3	160.10	1.911	1.192
SP 4	59.750	2.012	1.150
SP 5	494.35	2.021	1.445

4. Evaluating the Effect of Bacterial isolates on Plant Growth Promotion

The effect of five bacterial isolates on the plant growth of black gram, red flat bean and corn was evaluated at 15 days post inoculation. Among five strains, four strains except SP3 indicated the significantly increase in plant height compared with the uninoculated treatment (control). Strain SP2 and SP5 showed the maximum results on plant height, plant weight, root length and root weight in all selected plants.

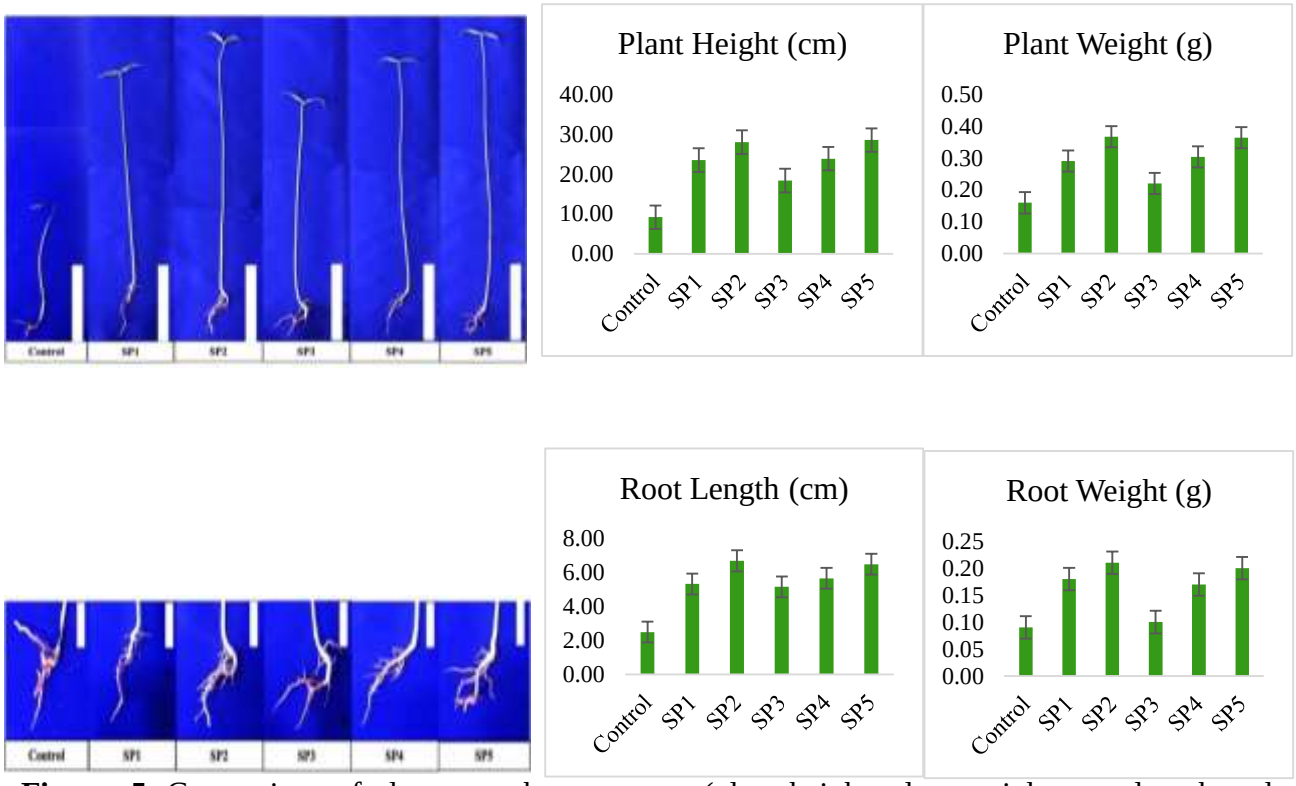

Figure. 5. Comparison of plant growth parameters (plant height, plant weight, root length and root weight) of five strains on black gram, inoculated with no-inoculation (control) at 15 days after sowing. The histograms at each treatment are significantly different at 15 days, P > 0.05 (t-test).

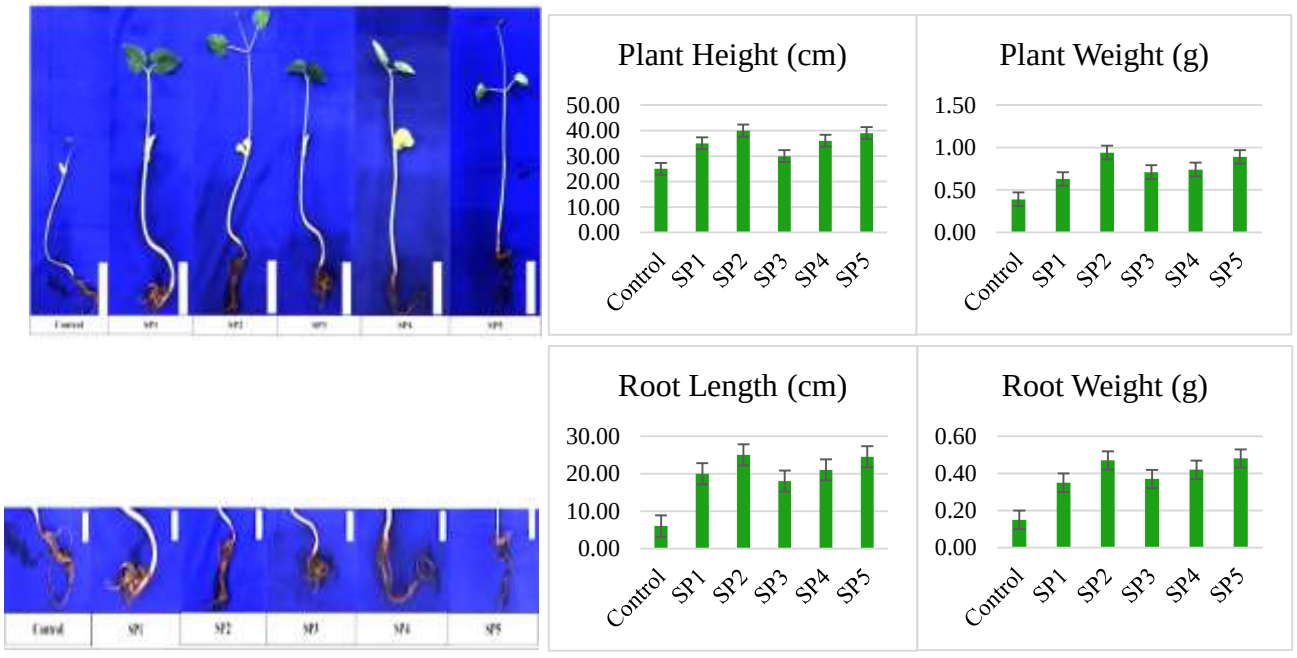

Figure. 6. Comparison of plant growth parameters (plant height, plant weight, root length and root weight) of five strains on red flat bean, inoculated with no-inoculation (control), at 15 days after sowing. The histograms at each treatment are significantly different at 15 days, P > 0.05 (t-test).

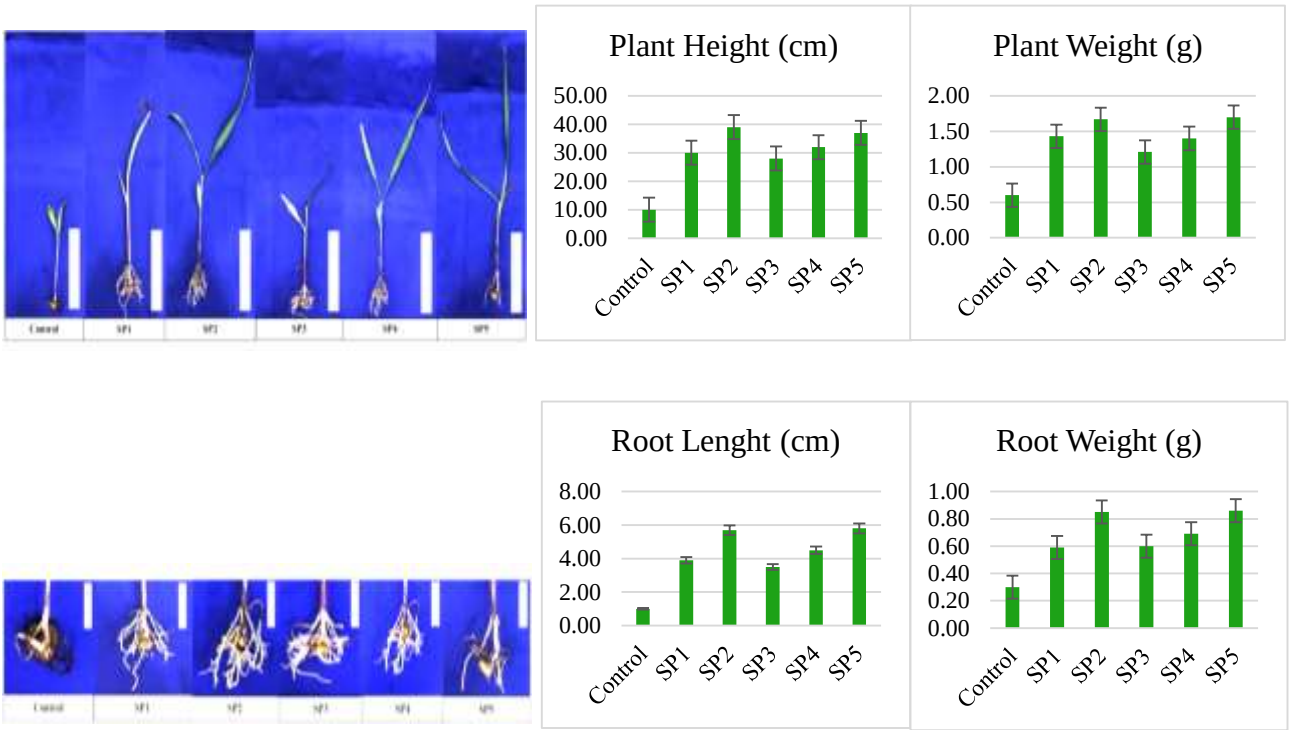

Figure. 7. Comparison of plant growth parameters (plant height, plant weight, root length and root weight) of five strains on corn, inoculated with no-inoculation (control), at 15 days after sowing. The histograms at each treatment are significantly different at 15 days, $P > 0.05$ (t-test).

Discussion and Conclusion

Plant growth-promoting bacteria (PGPB) are one of the most generalized findings of plant probiotic microbes (PPM) in soil, having *Bacilli* and *Pseudomonas* being the most common genera reported (Glick *et al*., 1997 and Hashem *et al*., 2019). In this study, five bacterial strains have been isolated from different soil samples and given named as SP1 to SP5. In the morphological characterization, all strains (SP1 to SP5) showed the circular shape, entire margin, raised elevation, smooth texture, and transparent opacity with the exception of opaque opacity in strain SP1. Microscopic observations demonstrated that strains SP1, SP2, SP4 and SP5 were Gram-positive with bacilli form and SP3 were observed as Gram-negative bacteria in the bacilli form.

In biochemical characterization, all isolates showed the positive results in catalase test, motility test, and TSI test. In contrast, the negative results of all bacterial strains were observed in indole test and VP test. The three isolates SP1, SP3 and SP4 indicated the negative result in citrate test and positive result were obtained in SP2 and SP5 which similar to the findings of Neihaya and Zaki (2018). For the MR test, SP1, SP2, and SP3 showed the negative results and SP4, and SP5 showed the positive results, which similar to study of Ambeng *et al.*, (2019). In urea test, SP1 and SP2 showed the positive result, and the negative result were observed in S3, S4 and S5. These are close agreement with the studies of (Anand *et al*., 2006 and Mandragutti *et al*., 2021). In Triple Sugar Iron Agar test, strains SP1, SP3 and SP4 indicated that the acid fermentation of dextrose, lactose, and sucrose. Strains SP2 taken place only dextrose fermentation and SP5 showed the absence of carbohydrates fermentation but the production of

H_2S gas was observed in both strains. Hiranmayee *et al*., (2023) described that TSI test provide carbohydrate fermentation and H_2S production, it can impact the plant growth. In the current study, the strains SP2 and SP5 showed the H_2S production which means it might be effectively promote the plant growth.

Demirkan *et al*. 2014 reported the positive results in motility, catalase and negative results in indole production from the *Bacillus* sp. strains isolated from soil samples. Similarly, many studies reported that the white colony color, smooth texture, Gram positive with rod shape in morphological characters as well as the positive results in catalase, and H_2S gas production and the negative results in MR, VP and citrate utilization test were identified as *Bacillus* spp. (Younes *et al*., 2012 and Neihaya and Zaki, 2018). The current study is closely related with their findings. For this reason, the current findings were assumed that isolated bacteria SP1, SP2, SP4, and SP5 might be *Bacillus* species except strain SP3. In addition to improvement of plant growth, nitrogen-fixing potential is an important attribute for plant growth promoting bacteria. In the observation of nitrogen fixing ability on a selective media, all isolated strains showed the growth of bacterial colonies after incubation at room temperature for 5-7 days which indicated the positive results of nitrogen fixation ability. Lihan *et al*., (2021) reported that all isolated PGPB strains, Gram-positive *Bacillus* sp. bacteria showed the positive reaction of growth on nitrogen free media and these results are the same with the current observation.

Moreover, high quality DNA from bacteria can promote plant growth by improving the plants resistance to stress, increasing soil fertility, and enhancing the fitness of plants (Parthasarathy, 2025). In the experiment of DNA extraction and determination of DNA concentration and purity by using Promega kit, all extracted bacterial DNA in the current study produce relatively high purity of DNA, the value of A_{260}/A_{280} indicated between 1.8 and 2.0 and the value of A_{260}/A_{230} showed the range under 2.0, which indicates the presence of contamination such as RNA, salt, and protein. However, strains SP2 and SP5 indicated the less contaminations in A_{260}/A_{230} ratio due to the results of nearly ~1.5. Similar observations were reported in the studies of Wilson, (1997), Courtois, *et al* (2001), and Tiago *et al*., (2015). Dwiyitno *et al*., (2018) reported that commercial kit/ Promega produced relatively high DNA quality ($A_{260/280}$ = 1.79-2.12) which indicated the similar results of present findings because strain SP5 showed the best DNA purity result.

The plant growth parameters including plant height, plant weight, root length and root weight of bacterial isolates indicated the positive effects on the selected plants (black gram, red flat bean and corn) at 15 days post inoculation. Among five bacterial strains, strains SP2 and SP5 indicated the maximum plant height, plant weight, root length and root weight in all selected plants, it might be SP2 and SP5 promote plant growth due to the H_2S production. Tozlu *et al*., (2012) described the application of *Bacillus megaterium* strain M-3 as biofertilizer improved the bean production. Similarly, the inoculation of corn with various *Bacillus* sp. significantly increases the shoot and root weight (Efthimiadou *et al*., 2020). In 2022, AlAli *et al*., stated that *Bacillus baekryungensis* DPM17 isolated from a non-agricultural area, and it improved the growth parameters such as root length, dry weight, and branching. Therefore, *Bacillus* spp. isolated form current study is closely agreement with their findings due to the achievement of highest effects on the growth of selected plants (bean and corn). Interestingly, strain SP3 was not identified as *Bacillus* although it showed the positive results on plant growth compared with control (uninoculated) plants. Moreover, the results of DNA indicated the best DNA purity ratio

with less impurities in SP2, and SP5 showed highest plant growth while other strains with some impurities in DNA result indicated moderate plant growth.

In conclusion, this study explores the importance of beneficial soil microorganisms which have the unique ability to promote the plant growth for agricultural productivity might be *Bacillus* species except one strain and preliminary molecular approaches such as DNA extraction and purification of all bacterial isolates indicated the high purified DNA with less impurities. These findings collectively point out that inoculation of plants with *Bacillus* spp. could be the excellent manner for speedy enhancement of plant morphological characteristics and might be play important role for sustainable agricultural development by reducing the use of chemical fertilizers. In future, it is remained to study the different PGPB traits of isolated bacterial strains and more efficiency test under greenhouse and field conditions as well as to clarify the role of PGPR as biofertilizers that exert useful consequences on plant growth and development.

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