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ISOLATION AND CHARACTERIZATION OF PHOSPHATE SOLUBILIZING BACTERIA FROM AGRICULTURAL SOILS OF AMHARA AND AFAR REGIONS ETHIOPIA.

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Abstract

Phosphorus (P) is one of the essential macro-elements that increase soil fertility and the growth and development of plants. However, phosphorus's complex occurrence makes it soluble and accessible. The objective of the present study was to isolate, characterize, and assess the phosphate solubilizing capacity of bacteria from agricultural soil samples of the Dupti wereda of Afar region and Deskua kebele, Kimir-Dingay district in the south Gondar Zone. This study was conducted at the Cellular and Microbial Laboratory, Institute of Biotechnology, University of Gondar, Gondar, Ethiopia.

Isolated phosphoramide solubilizing bacteria were characterized morphologically and biochemically. A total of ten phosphate-solubilizing bacteria were isolated and were coded as DU1, DU2, DU3, DU4, DU5, DU6, DE1, DE2, DE3, and DE4. Out of ten isolates, eight were gram-positive, and two were gram-negative. The phosphate solubilization *efficiency of isolates was* identified based on the formation of a clear zone around PVK media and liquid broth containing calcium triphosphate. Among the isolates DU5, DE1, DE2 and DU1 have been observed to be the maximum solubility index. When the solubilization efficiency of isolates was observed in liquid broth, *the highest P release was recorded by isolates DE1 followed by* DU5, DE2 and DE4. Based on solubilization efficiency and stress-tolerant capability, only six isolates were selected for pot experiments in the faba bean and wheat varieties. Isolate PSB DU5 showed the best effect on shoot length (71.66 ±15.27), root length (12.66 ±3.7), leaf number (39±4.35), nod number (13.3±0.57). Isolates PSB DU1, PSB DE1, and DE2 showed second and third-best

effects on the shoot length of Dosha faba bean growth, respectively. Similarly, PSB DU3 significantly increased root length. When the impact of the isolates is compared based on their shoot length of the inoculated Eliodoro wheat variety, isolates DU1 and DU5 were observed to be the most effective in enhancing plant growth for shoot length.

Keywords: Phosphorus, phosphate solubilizing bacteria, solubility index**.**

Introduction

In Africa, the causes of land degradation and declining soil fertility are intricately intertwined and cyclical. In densely populated places like southern Sudan, Ethiopia, and western Kenya, land fertility has considerably dropped and depleted as a result of continuous usage without external inputs (Hoekstra and Corbett, 1995). Because of continual farming to meet the food needs of the constantly expanding global population, soil fertility is now fluctuating. As a result, forests are being lost to the growth of agricultural land.

One of the fundamental macroelements necessary for the growth and development of plants is phosphorus (P). Next to nitrogen, it is a key nutrient that limits development. According to Mengel and Kirkby (1978), the majority (95–99%) of phosphorus in soil is found as insoluble organic and inorganic phosphates, which are slowly released into the soil. According to Tenzing et al. (2016), inorganic phosphate is present in the ground in a variety of complex forms, including ferric phosphate, calcium phosphate, and aluminum phosphate. Phosphorus is necessary for the growth of roots, the strength of stalks and stems, the development of flowers and seeds, and crop maturity. However, its complex shape renders it insoluble and inaccessible to plants. Chemical fertilizers boost agricultural yield (Richardson, 2001), but they also create severe environmental effects.

Ethiopia is a well-known agricultural nation, and significant efforts are being undertaken to raise crop output to feed the expanding human population. Ethiopia's population is expanding quickly, increasing the need for food. In order to ensure food security, there is a significant need for agricultural land and contemporary agricultural inputs. However, importing chemical fertilizers costs Ethiopia a lot of money. In addition to their negative effects on the environment, chemical fertilizers are expensive due to their transaction and shipping expenses. According to Minten et al. (2013), 64–80% of these pricing variations may be attributed to transportation expenses. By introducing bio-fertilizers such as phosphate-solubilizing bacteria obtained from local habitats, expenses could potentially be minimized, and Crop yield and soil fertility can both be enhanced.

Potential phosphate-soluble bacteria (PSB) for use as biofertilizers can take the place of chemical fertilizers, save money on the cost of importing chemical fertilizers, and preserve the soil's acidity and environmental safety that come with chemical fertilizer use. Additionally, these PSBs may be employed in farming systems and have a big impact on raising farmers' output to feed Ethiopia's expanding population. Thus, the nation as a whole, farmers, scientists, politicians, and everyone are the main beneficiaries of such a study.

The objective of the present study was to isolate and characterize phosphate solubilizing bacteria from agricultural soil samples of South Gondar (Kimr-Dingay, Amhara region) and Dupti kebele (Afar region) and the promoting potential of a legume (Faba bean) and non-legume crop (wheat).

Materials and Methods

Study area

This study was conducted in Dupti Kebele in the Afar Region of Ethiopia. It is located at the eastern end of the Afar Region Capital, Semera, Ethiopia. This district is characterized by high humidity and temperature, which range between 27°C to 45°C. The district has one urban and eight rural peasant associations (PAs), of which three (Alasa Bolo, Humodoyeta, and Mego) are totally agro-pastoralist PAs. The elevation of the district is between 280–850 meters a.s.l., with an average annual temperature of 120 mm. The land coverage of the district is about 1926 km ^{2,} and the district is known as Lake Abbi, which is the final destination for the Awash River (ARFEB, 2007). Kimir-Dingay district is one of the study areas included in this study. It is located in the South Gondar Administrative Zone of the Amhara National Regional State, Ethiopia. It is about 100km north of the Regional Capital- Bahir Dar and 660 km north of Addis Ababa. The total area of the Woreda is 109,925 hectares. According to CSA (2007), Deskua kebele is one of the kebele from this Woreda and is located at Latitude: 12° 00' 0.00" N, Longitude: 38° 00' 0.00" E.

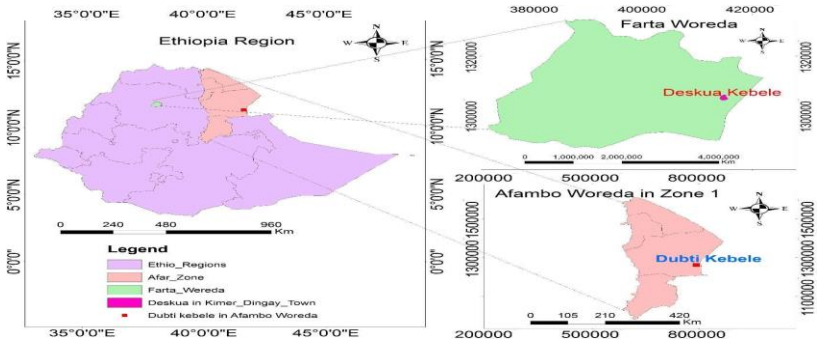

Figure 1: Map of the study area

Sampling site selection

Prior to the opening of soil profiles, personal field observation of the selected agricultural study areas was carried out to determine which specific areas should be selected as representative sites of the study area based on salinity, acidity, and high temperature. The study sites were selected based on the general fact that areas incorporated as study sites are believed to be affected by different environmental factors such as salinity and acidity. Moreover, the sampling site selection was done based on the cropping history of lands and the possibility of occurrences of phosphate-solubilizing microbes. Accordingly, two representative soil sampling sites for each study site with similar cultivation histories were selected randomly.

Study design and study period

The study was conducted from October 2019 to June 2020. An experimental design was used to conduct this study. Pots were arranged in a complete random block design (CRBD).

Soil sample collection

In each sampling site, a 100g surface soil sample (0 to 30 cm depth) was collected from Deskua kebele (acidic soil) and Dupti kebele (saline soil and high temperature). Each surface soil sample was collected with sterilized plastic bags from a plot size of 25m by 25m from the land area

represented by the respective soil profile. The representative study sites are (1)Deskua kebele, Kimrir-Dingay district, South Gondar Zone, Farta wereda), which is believed that this area is affected by acidity; (2) Dupti kebele, the area around Afar regional state capital city, Semera which is assumed to be saline and experiences high temperature. From these sites, soil samples were collected with sterilized plastic bags and transported to the University of Gondar for phosphate solubilizing bacteria isolation, screening, and characterization.

Soil sample analysis

In this study, physicochemical analysis of the soil samples, such as pH, CEC (cation exchange capacity), electrical conductivity, and macro-elements (total nitrogen and organic matter, phosphorus, potassium, sodium, magnesium, and calcium) were analyzed. Soil analysis for the mentioned physicochemical parameters was done at the Gondar Agricultural Research Center according to (Richards, 1954). The availability of phosphorus (Olsen *et al*., 1954), potassium (Knudsen *et al*., 1982), total nitrogen (Bremner, 1965) and organic matter (Smith and Weldon,1941), sodium, magnesium, and calcium analysis were done based on standard soil analysis methods (Bashour and Sayegh, 2007; Hialan and Unal, 1965).

Isolation of Phosphate solubilizing bacteria

One Gram of soil from each sample was serially diluted from 10^{-1} up to 10^{-7}. From dilutions of 10^{-5}, 10^{-6}, 10^{-7} 0.1 ml of suspension was taken and inoculated onto Pikovskayas's agar medium according to Pikovskaya (1948). The bacteria were isolated and screened on Pikovskayas's medium, which consists of (Glucose 10 g, Magnesium sulfate 0.1g, Ferrous sulfate trace, Manganese sulfate trace, Tricalcium phosphate as a P source, agar 15 g, Distilled water 1L, pH was adjusted to 7 before sterilization, followed by pour plate technique and incubated at 30 ^{0}C for 48 hr., discrete colonies showing halo zones were picked up and subcultured on Nutrient agar slants and preserved. The isolates were also characterized based on the colony morphological characteristics (*i.e.*, shape, color, and elevation margin of the bacterial colony) on their respective plate and gram staining (Karpagam and Nagalakshmi,2014). Further identification of isolates was done by using various biochemical tests.

Biochemical Characterization of Phosphate solubilizing bacteria

Growth on Glucose Peptone (GP) Agar medium

A glucose peptone agar medium was used to identify the ability of the bacterial isolates to utilize glucose as the sole carbon source. The GPA medium (containing 40g glucose, 5g peptone, and 15 g agar in 1L water) was prepared, and the pH was adjusted to 6.7. Then 0.1 ml of bacterial suspension was inoculated. After inoculation, the petri dishes were incubated at 30^{0}c for four days. The presence of growth was recognized as positive for glucose utilization (Singh et al., 2008).

Congo red Yeast Extract Mannitol Agar (CRYEMA) medium

Yeast extract mannitol agar (YEMA) medium containing 2.5% congo red dye per 1L of YEMA solution was prepared and adjusted at a pH of 6.7. Then, 0.l ml of bacterial suspension was spread

on the plate, and the inoculated plates were incubated at 30^0c for four days. Then, the growth of bacteria was positive for this test (Pervin et al., 2017).

Methyl Red and Voges Proskauer test

In test tubes, a glucose phosphate broth medium was made up of 5 grams of glucose, 5 grams of peptone, and 5 grams of K_2HPO_4 in 1 liter of distilled water. After adding 0.1 ml of bacterial suspension to the tubes, they were incubated at 300 °C for three days. Next, five drops of the indicator (0.1 g methyl red in 300 ml of ethanol + 200 ml of distilled water) were added to each culture tube for the methyl red (MR) test. Additionally, five drops of the indicator (0.6 ml of 5% Alfa naphthol in absolute alcohol and 0.2 ml of 40% potassium hydroxide) were added for the Voges-Proskauer test. This test determines the bacteria's ability to metabolize pyruvic acid. (Harden,1906).

Triple sugar iron (TSI) agar test

Triple sugar iron agar media consisted : (of 1 g dextrose, 3 g yeast extract, 15 peptone, 3g beef extract, 5g NaCl, 10 g lactose, 10 g sucrose, 0.2 g ferrous sulfate, 0.3 g sodium thiosulfate, 0.24 g phenol red, 15 g agar per liter of distilled water). It was prepared in test tubes, and the final pH was adjusted to a pH of 7. Each test tube was inoculated with 0.1ml of bacterial suspension and incubated at 35ºC for three days. The color of the butt of the slant and the production of acid, CO_2, and hydrogen sulfide were recorded to determine the capability of bacterial isolates to utilize various carbohydrate sources (sucrose, glucose, lactose (Hajnaa, 1945).

Simmon's Citrate Agar

This test was performed to identify the organism's capability of using citrate in Simmons Citrate Agar as the sole carbon source and metabolizing the ammonium salt in the medium. The increase of pH of the medium and development of color change in the bromothymol blue indicator was considered positive for the utilization of citrate and growth of bacterial isolate on Simmons Citrate Agar (Duquesne et al.,2007).

Urease production test

This test is used to check the ability of an organism to produce an exoenzyme called urease, which hydrolyzes urea to ammonia and carbon dioxide. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created, and the media turns pink (Aneja, 1996).

Indole Test

The indole test medium (20g tryptone, 5g lactose, 2.75g dipotassium hydrogen phosphate, 2.75g dihydrogen potassium phosphate, 5g sodium chloride, and 7g sodium lauryl sulfate in 1L distilled water) was used and prepared in test tubes. Then, each tube was inoculated with 0.1ml of aliquot bacterial isolates inoculated into the test tube and incubated for three days at 30^0c. The ability of the isolate to split indole from the amino acid tryptophan was observed by adding five drops of Kovac's reagent to the culture broth (Cheesbrough, 1993).

Motility test

For this test, a semi-solid nutrient agar was prepared with 0.5% agar concentrate in test tubes. Then, a loopful of bacterial isolate was inoculated using the agar stab method to the center of the tube from surface to bottom using a stab needle. All inoculated tubes were incubated at 30°C for three days. Diffused growth originating from the inoculated center zone to the periphery of the tube was considered positive for motility. Meanwhile, growth only on the inoculated line was labeled as non-motile (Kim and Rhee,2012).

Catalase test

This test was performed to study the presence of catalase enzyme in phosphate solubilizing bacteria (McFadden, 1980) H_2O_2, which would be converted into H_2O and O2 (Baird-Parker,1974). Determination of the isolates' phosphate solubilizing capacity based on PVK culture media pH change

The phosphate solubilizing ability of the isolates was determined in terms of pH change in 7,10, and 15-day intervals of bacterial inoculated PVK culture media.

Phosphate solubilizing ability assay

As the plate assay is not considered a reliable method in determining a strain as phosphate solubilized, the pure cultures were further screened in a liquid medium containing Ca_3 $(PO_4)_2$ as an insoluble P source. The isolated strains were grown in a liquid medium and were shaken at 30°C for 20 h. sterile water-inoculated medium was treated as a control. Three Erlenmeyer flasks for statistical replication were used to incubate in the dark on a shaker at 30°C for three days. The supernatant of the medium was used to assess P released into the solution. Phosphorus in the culture was determined by the molybdenum blue method with a spectrophotometer at a wavelength of 410nm (Watanabe and Olsen, 1965).

Estimation of phosphate solubilization efficiency

The solubilization efficiency of PSB to solubilize tricalcium phosphate on Pikoviskayas agar medium was determined in terms of solubilization index (PSI). PSI was calculated by measuring the colony diameter and halo zone diameter using the following formula (Edipremono et al.,1996).

$$Psi = \frac{\text{colony diameter+halo zone diameter}}{\text{Colony diameter}}$$

Screening of Bacterial isolates for their stress tolerance capability

Screening for NaCl salt tolerance

Screening for different salt stress tolerance phosphate solubilizing isolates was tested on Nutrient broth with salt (5%, 10%, 15%, and 20%) growth which was repeated three times. Further, their salt tolerance was evaluated by growing each of the isolates in solid culture at 28ºC for 48 hours. The growth of isolates at different concentrations of NaCl was then compared with the control (Mohan et al., 2017).

Screening for Temperature tolerance

Screening for temperature tolerance viz all efficient isolates to promote plant growth were tested at different temperatures (25^0c, 28ºC, 37ºC, 40ºC, 45ºC) for two days. A loop full of the culture of each isolate was inoculated in a 5ml broth tube and incubated at 28^0C for 48-72 hours. After this, 100µl broth cultures from each tube were spread on agar plate media and allowed to grow for 48-72 hours at 28^0C and then determine whether to grow well or not (Chaiharn and Lumyong,2009).

Screening for pH tolerance

The pH stress tolerance of all the isolates was tested on a solid media plate with pH (5.0, 6.0, 7.0, 8, and 9) by inoculating from log phase culture and incubating at 28^0c for 48-72 hours(Pal,1998).

Pot experiment

The phosphate solubilizing and growth-promoting isolates were determined using the Dosha faba bean cultivar and Elio Doro weight variety collected from Amhara Regional Agricultural Research Institute, Gondar, as a plant material. Then forty-eight Plastic pots, which measure 24.5 in diameter and 20.5 cm depth, were bought from the local market. These pots were surface sterilized with 95% ethanol and rinsed with clean water. The soil sample for the pot experiment was collected on the Tewodros campus, University of Gondar, Gondar. These soil samples were sterilized at 100ºC for 3 hours using a dry, hot oven(Hoben and Samasegarian, 1994; Shamseldin, 2012; Vincent, 1970). Each plastic pot was filled with 7 kg of sterilized soil. Seeds were surface sterilized with sodium hypochlorite solution (0.5%) for three to four minutes and then washed in clean water for planting. Then, each plastic pot was inoculated with surface sterilization, and four seeds of Dosha faba bean cultivar and eliodoro weight variety were 4cm apart. After ten days of planting the cultivars, 300 bacterial isolate suspensions (standardized to 10^5 to 10^6 cfu ml-1) and nutrient broth media were inoculated in each pot. The media alone was used as a negative control, whereas chemical fertilizer (DAP) was used as a positive control. Inoculated pots were arranged in a completely randomized design. The pots were watered twice per week. The root and shoot growth were evaluated on the 45^{th} and 60^{th} day of inoculation. The uprooted plantlets were also used to evaluate biomass and dry weight (Araújo et al., 2012)

Data analysis

The data were analyzed using the SPSS software package version 22. Means and standard deviations of the triplicates analysis were calculated by one-way analysis of variance (ANOVA) to determine the significance differences between the means followed by Duncan's multiple range test ($P \leq 0.5$)

RESULTS

Physicochemical analysis of soil samples

The physicochemical analysis of soil samples revealed that the two study areas have significantly different physicochemical profiles, as indicated in Table 1. Phosphorus analysis indicated that the availability of phosphorus (p/ppm) in the soil samples is almost similar, except for Deskua kebele.

The pH analysis of the study area soil indicated that the pH value of soil samples ranged from 6.7 to 8.24, recorded by Deskua and Dupti Keble, respectively. Similarly, electrical conductivity (EC)

analysis of the study area showed that soil samples have significantly different electrical conductivity, with lowest value of 0.05 obtained from the Deskua Kebele sample and the highest value, 1.13, recorded from the Dupti Kebele soil sample.

Regarding macro-elements and organic matter analysis, the soil samples have different levels of organic matter and microelements. The organic matter content ranged between 1.29-4.17% recorded from Dupti and Desekua soil samples, respectively. Likewise, the concentration of macro-elements such as calcium, potassium, sodium, and magnesium is significantly different, as shown in Table 1.

Table 1: Physicochemical analysis of soil samples

	Soil analysis parameter									
Sampl	**pH**	**EC**	**OM**	**Available**	**TN**	**CE**	**Ca^{++}**	**K^{++}**	**Na^{+}**	**Mg^{+}**
Du1	8.24	0.69	1.44	2.87	0.07	43.87	30.92	2.02	4.63	5.56
Du2	8.6	0.22	1.65	2.37	0.08	40.66	31.03	1.39	4.34	3.75
Du3	8.55	0.2	1.37	2.08	0.07	41.09	30.17	2.15	4.51	4.17
Du4	8.22	0.86	1.80	3.49	0.09	45.37	30.50	2.20	5.90	6.10
Du5	7.97	1.13	1.29	2.20	0.06	44.30	29.10	1.93	4.95	6.74
Des1	6.74	0.07	4.17	5.14	0.21	43.44	24.18	2.06	1.63	1.63
De2	7.43	0.05	1.73	2.76	0.09	38.95	23.75	0.82	1.89	4.17
De3	7.3	0.2	2.37	2.59	0.12	44.9	18.3	1.32	1.8	9.31

Isolation and characterization of isolates

In the present study, ten isolates have been isolated. Morphologically, most of the isolates were smooth, circular, transparent, spore formers, and white colonies, as shown in Table 2. Three of the isolates designated as DU1, DU5, and DU6 are found non-spore formers. In contrast, the remaining seven isolates are found in spore formers. As revealed in Table 2, all of the isolates are found smooth in their colonies' texture. All of the isolates of the colony were transparent.

Table 2: Morphological characterization of isolates

Isolates	**Colony color**	**Colony texture**	**Colony Shape**	**Cell shape**	**Appearance**	**Transparency**	**Spore formation**

DU1	White	Smooth	Circular	Short rode	Raised	Transparent	No spore
DU2	White	Smooth	Circular	Long rode	Slightly raised	Transparent	Spore
DU3	White	Smooth	Circular	Long rode	raised	Transparent	Spore
DU4	White	Smooth	Circular	Short rod	Raised	Transparent	Spore
DU5	Yellow	Smooth	Circular	Short rod	flat	Transparent	No spore
DU6	Pink	Smooth	Circular	filamentous	Raised	Transparent	No spore
DE1	White	Smooth	Circular	Long rod	Slightly raised	Transparent	Spore
DE2	Yellow	Smooth	Circular curlyEdge	Short rod	Flat	Transparent	Spore
DE3	White	Fried egg	Circular	Long rode	Flat	Transparent	Spore
DE4	White	Smooth	Circular	Short rode	Raised	Transparent	Spore

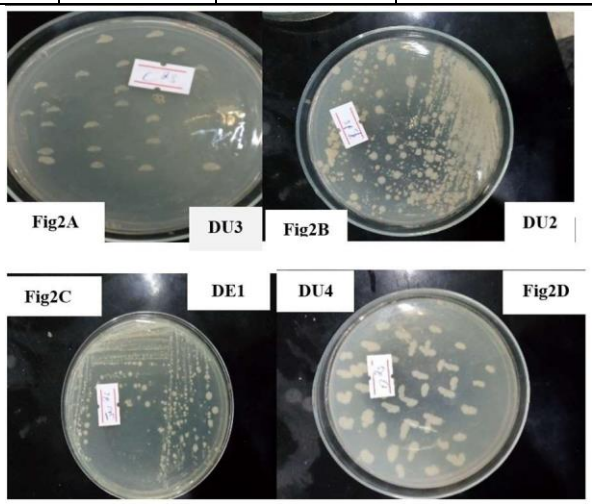

Figure 2: Morphological characterization.

Gram staining and Biochemical characterization of bacterial isolates

Gram-staining characterization of bacterial isolates

As shown in Figure 2, except for DU1 and DU5 all isolates were Gram's positive. The isolates were further characterized by a series of biochemical tests, as shown in Table 3. Biochemical characterization showed that all isolates are positive for starch hydrolysis and catalase test. However, they are all negative for the indole and oxidase test. Except for DU1 and DU4, all isolates are urease-positive. In the motility test, all are motile except DU6 and DE3. Based on the biochemical test result, the isolates were identified as general bacillus and general Pseudomonas.

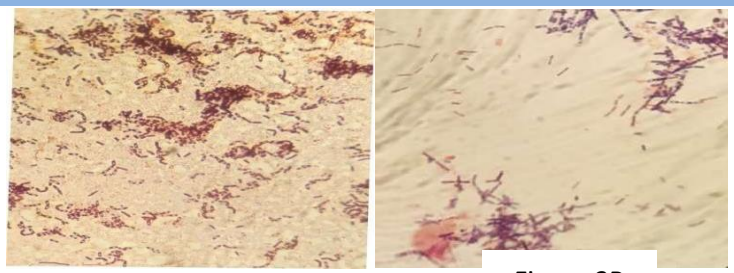

Figure 3B

Figure 3A and 3B: Gram's staining reaction r.

Table 3: biochemical characterization of isolates

S.No	Characteristics	DU1	DU2	DU3	D U4	DU5	DU6	DE1	DE2	lDE3	DE4	DE5
1	Gram reaction	-	+	+	+	-	+	+	+	+	+	+
2	Citrate test	+	+	-	+	+	+	-	-	+	-	-
3	Motility	+	+	+	-	+	-	+	+	+	+	-
4	Urease test	-	+	+	-	+	+	-	+	+	+	+
5	Glucose peptone agar	+	+	+	+	+	-	+	+	+	+	+
6	Catalase	+	+	+	+	+	+	+	+	+	+	+
7	Indole test	-	-	-	-	-	-	-	-	-	-	-
8	Starchhydrolysis	+	+	+	+	+	+	+	+	+	+	+
9	Manito fermentation	+	-	+	-	-	-	-		+	+	+
10	Oxidase test	-	-	-	-	-	-	-	-	-	-	-
11	Vogesproskaures test	-	+	+	-	-	-	-	-	-	-	-
12	Methyl red	-	-	+	+	-	-	+	-	-	-	-
13	Lactose fermentation	+	-	+	+	+	+	+	+	+	+	+
14	Sucrose fermentation	+	+	+	+	+	+	+	+	+	+	+
15	H_2S production	-	-	-	-	-	-	-	-	-	-	-

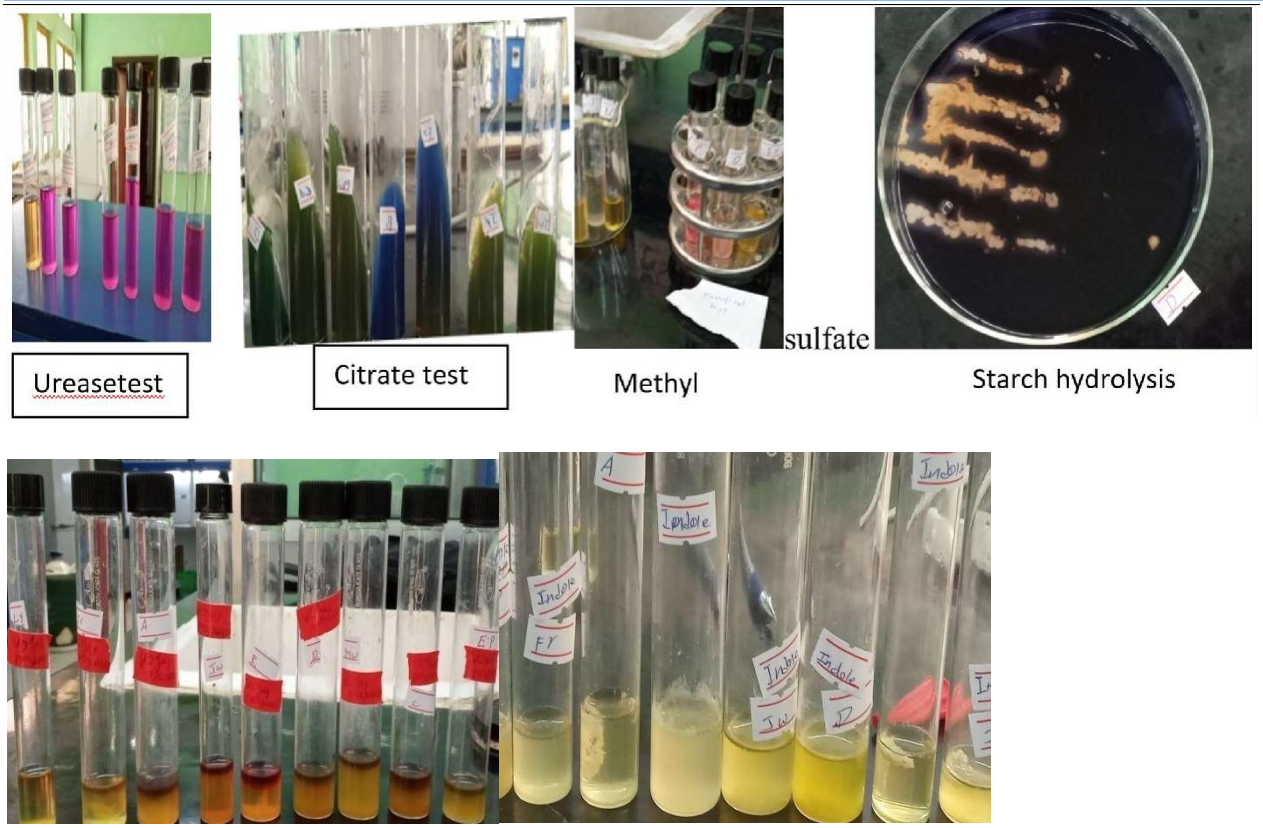

Figure 4: Biochemical characterization of isolates

3.4. Phosphate solubilizing capability assay

3.4.1 determination of the isolates' phosphate solubilizing capacity based on PVK culture media pH change.

As shown in Table 4, the pH of the medium was initially neutral (pH=7). However, as the time of incubation increased, the pH of the broth media decreased from the initial. pH after inoculating with the isolates. Based on the observation, the pH of the media inoculated with isolate DU2, DE2, DU1, DU3DU4, and DE3 were the lowest after fifteen days of incubation. In addition, the pH of the media inoculated with isolate DU6, DE3 DU5, and DE4, DE1 were next to DU2, DE2, DU3, DU4, and DE3 after fifteen days of incubation. The observed result has indicated that there was phosphate solubilization due to organic acid secretion.

Table 4*:*: Initial and final pH of PVK liquid medium after 7, 10, 15 days of inoculation

Isolates	pH of media before inoculation	pH of bacterial inoculated pvk media after 7 days	pH of inoculated pvk media after 10 days	pH of inoculated PVK media after 15 days
DU1	7	4.69	4.5	3.85
DU2	7	4.79	3.75	3.5

DU3	7	4.6	4	3.81
DU4	7	4.85	4.53	3.87
DU5	7	4.9	4.5	4.00
DU6	7	4.8	4.5	3.92
DE1	7	4.8	4.32	4
DE2	7	5.2	5	3.72
DE3	7	4.64	4.2	3.93
DE4	7	4.77	4.2	4
Negative control	7	6.8	6.78	6.7

5.4.2. Phosphate solubilization index (Psi) determination on PVK culture media

Out of the ten phosphate solubilizing bacteria, six from Dupti and four from Deskua, six of the isolates are found to have higher phosphate solubilizing abilities on Pikovskaya's agar plate, which were selected for further evaluation to enhance plant pot experiment.

All six isolates showed clear halo zones around their colonies. The isolates designated as DU1, DU3, DU4, DU5, DE1, and DE2 showed maximum solubilization index in Pikovskaya's agar media, respectively. The isolates were grown on PVK media plates using sterilized cotton swap, and the plates were incubated at 28^0c for seven days. After that phosphate solubilization index was calculated as shown in Table (7.). DU5, DE1, DE2 have almost similar (6 mm) solubility indexes.DU3 and DU4 have 4.5 and 5 mm respective solubility index

Similarly, the isolates formed a clear (halo) zone diameter of between 10mm and 15mm, and the maximum clear zone diameter of 15mm was observed in DU5 followed by 14mm in DU3 and 12mm in DU4, and the least clear zone was obtained in DE1 and DE2.

Table 5: Phosphate solubility index mean of isolates

PSB isolates	**Hole zone diameter (mean)**	**Solubility index**
DU1	17	5.6±*0.21*
DU2	11	*3.6 ± 0.13*
DU3	14	4.5±*0.12*
DU4	12	5± 00
DU5	15	6± 00
DU6	12	*3.71 ± 0.19*
DE1	10	6±0
DE2	10	6±0
DE3	5	*2.69 ± 0.07*
DE4	5	*2.01 ± 0.10*

Figure 5: Show clear zones of isolate

3.4.3. Spectrophotometric Determination **of phosphate solubilization ability**

The results of insoluble phosphate $(Ca_3PO_4)_2$ solubilization by different isolates are shown in Table 8. All bacterial isolates used in this test are solubilized with a greater amount of tricalcium phosphate over an un-inoculated control. The highest amount of solubilization is recorded by bacterial isolates DE1, DU5, DE2 DU6, and DU1. The result showed that PSB isolates isolated from extreme environments can solubilize insoluble phosphate well.

Table 6: Phosphate solubilization (mg/ml)

Isolates	Solubilization mg/ml (means ± SD)
DU1	0.584 ± 0.37
DU2	0.25 ± 0.04
DU3	0.31 ± 0.11
DU4	0.54 ± 0.34
DU5	1.01 ± 1.02
DU6	0.63 ± 0.84
DE1	2.16 ± 1.96
DE2	0.907 ± 1.25
DE3	0.55 ± 0.27
DE4	0.7 ± 0.9
Control with media	0.025 ±0.01

3.5. Screening bacterial isolates for different stress tolerance

3.5. 1. Bacterial growth at different pH

All the isolates were screened for their capacity to grow under a wide range of pH. Accordingly, isolates were analyzed using pH ranging from pH-5 to pH-9. As shown in Table 4, seven of the isolates were found to grow in a wide range of pH, ranging from pH-5 to pH-9. However, isolate DU6 is found to grow near neutral and slightly alkaline pH (pH-7 and pH-8). Whereas isolate DE3

and DE4 are observed to grow under acidic and neutral pH but are unable to grow under high saline conditions.

Table 7: Bacterial growth at different pH

BACTERIA	pH-5	pH-6	pH-7	pH-8	pH-9
DU1	+	+	+	+	+
DU2	+	+	+	+	+
DU3	+	+	+	+	+
DU4	+	+	+	+	+
DU5	+	+	+	+	+
DU6	-	-	+	+	-
DE1	+	+	+	+	+
DE2	+	+	+	+	+
DE3	+	+	+	+	-
DE4	+	+	+	+	-

3.5.2. Bacterial growth at different salt concentrations

All of the isolates were screened for their salt tolerance capacity by growing under different concentrations of NaCl on PVK medium. Accordingly, all the isolates were grown on PVK medium containing 5% and 20% of NaCl. Six of the isolates, four isolates (DU1, DU3, DU4, and DU5) from the Dupti kebele sample, and two isolates (DE1 and DE2) from the Deskua kebele sample were found to be able to grow in 15% and 20% NaCl containing media as shown in table 5.

Table 8: Salt tolerance of isolates

Isolates	5% NaCl	10% NaCl	15%NaCl	20% NaCl
DU1	+	+	+	+
DU2	+	+	+	+
DU3	+	+	+	+
DU4	+	+	+	+

DU5	+	+	-	-
DU6	+	+	+	-
DE1	+	+	+	+
DE2	+	+	+	+
DE3	+	-	-	+
DE4	+	+	+	+

3.5.3. Bacterial growth at different temperatures

To screen mesophilic and thermophile bacterial isolates, all the isolates were screened for their capacity to resist different temperatures ranging from 25^0C to 45^0C. As a result, all the isolates were able to resist as high as 45^0C, as revealed in Table 6.

Table 9: temperature tolerance

Isolates	25^0C	28^0C	37^0C	40^0C	45^0C
DU1	+	+	+	+	+
DU2	+	+	+	+	+
DU3	+	+	+	+	+
DU4	+	+	+	+	+
DU5	+	+	+	+	+
DU6	+	+	-	+	+
DE1	+	+	+	+	+
DE2	+	+	+	+	+
DE3	+	+	+	+	+
DE4	+	+	-	+	+
DE5	+	+	+	+	+

3.6. The effect of bacterial isolates inoculation on *faba bean* growth

3.6.1. The effect of bacterial isolates inoculation on *faba bean* growth after 45 days

When the effect of the isolates was compared based on the shoot length of the inoculated *faba bean*, isolate DU3 was observed to be the most effective in enhancing the plant growth as compared with the remaining isolates and the negative control, as shown in Table 10. As statistically revealed (P=0.01), it has been observed that there is a significant difference among the isolates to enhance the growth of *faba bean* with respect to shoot length. In contrast, Isolate DE2 is the least effective in promoting the growth of *faba bean* shoot.

As the effect of the isolates is compared based on the leaf number of inoculated faba bean the isolate DU5 (20±1.7) has been the most effective as compared with the remaining isolates, the positive control and negative control. As statistically revealed p= 0.024 it has been observed that there is a significant difference among the isolates to enhance the growth of *faba bean* with respect to leaf number. Whereas the isolate DU1 has the least effect on promoting the growth of leaf number. When comparing the effects of isolates based on the node number of inoculated faba bean DU1, DU4, DU5, DE1, DE2 have similar effects with negative control. There is no significant difference among the isolates to promote the growth of node number of faba bean, whereas DU3 has the least effect to promote the growth of node number of faba bean with negative control and other isolates.

When the effect of isolates is compared based on their biomass increasing on inoculated faba bean, DU3, DU4, DU5, DE1, DE2 isolates are effective in enhancing the faba bean plant growth with respect to biomass compared with negative control. As statistically revealed p= 0.48 it has been observed that there is no significant difference among the isolates DU3, DU4, DU5 DE1, and DE2. Whereas the isolate DU1 has the least effect on promoting the growth of faba bean biomass as compared with negative control and the other isolates.

When the effects of isolates were compared based on increasing dry mass of inoculated faba bean, isolate DE 1 was observed to be effective in enhancing the dry mass of faba bean as compared with other isolates and negative control. Whereas the isolate DE2 is least effective in increasing dry mass of faba bean as compared with other isolates it has a similar effect with negative control.

Table 10: Effect of PSB isolates on different growth parameters in Dosha faba bean under green house condition after 45 day (Mean and STD of faba bean)

Treatment	DU1	DU3	DU4	DU5	DE1	DE2	+Ve control	-ve control	P≤0.05
Shoot length (cm)	44 ± 2	55.3 ± 2.08	42.67 ± 11.84	42 ± 2	41.67±2.08	39.33±3.5	52.33±2.08	34±2.64	0.01

Root length in (cm)	9.166 ± 1.04	7.5 ± 2.17	10.3±1.52	7.166±0.288	7.166±0.288	9.33±4.6	11.83±0.288	8.5±1.8	0.85
Leaf number	15.66± 1.52	16.33±0.57	18±4.58	(20±1.7)	19.±3.6	17±1	17.66±1,52	12±10	0.024
Node number	6.66±0.57	4.66±0.57	6.33±0.577	6±0.0	6.33±1.15	6.33±0.77	8±1.0	6.33±0.57	0.004
Biomass	4.79±66.8	9.733±3.21	9±0.64	9.2±3.46	9.76±3.08	9.5±2.4	14.33±1,52	8±2	0.47
Dry mass	3.16± 1.25	3.166± 1.25	3.1±0.38	3.166± 0.76	4.03±1	2.96±0.3	8..2±1.088	2.63±0.	0.85

Figure 6: Effects of PSB isolates on dosh faba bean

3.6.2. The effect of bacterial isolates inoculation on *faba bean* growth after 60 days

As compared to the effect of the isolates based on their shoot length of the inoculated *faba bean, the* isolateDU5 was observed to be the most effective in enhancing the plant growth as compared with the remaining isolates and the negative control as shown in Table 11, As statistically revealed (P=0.012) it has been observed that there is the significant difference among the isolates to enhance the growth of faba bean with respect to the shoot length. Whereas the isolate DU3 has—the least effect on promoting the growth of faba bean shoot length. When the effect of the isolates was compared based on the root length of the inoculated faba bean, isolate DU3 was most effective in enhancing faba bean growth as compared with the remaining isolates and negative control. As statistically revealed p =0.12 it has been observed that there is no significant difference among the isolates to enhance the growth of faba bean plant with respect to root length.

As compared to the effect of the isolates based on leaf number growth of inoculated faba bean, the isolates DU5 and DE2 were observed to be the most effective as compared with the remaining isolates and the positive control and negative control. Statistically revealed p= 0.03, it has been

observed that there is a significant difference among the isolates to enhance the growth of faba bean with respect to leaf number, whereas DU4 is the least effective to promote the growth of leaf number of faba bean. When the effect of isolates is compared based on the node number of faba bean, the isolates DU1, DU3, DU5, and DE1 were observed to be effective as compared with positive control and negative control. When the effect of isolates is compared based on flower number of faba bean, the isolate DU1 and DU5 were effective as compared with the remaining isolates and positive control and negative control. Meanwhile, the isolate DU4 is the least effective as compared with the remaining isolates. When the effect of the isolates is compared based on the shoot biomass of the inoculated faba bean, isolate DU1 was observed to be the most effective in enhancing faba bean shoot biomass as compared with the remaining isolates and positive control and negative control. Whereas the isolate DU4 has the least effect on promoting increasing shoot biomass of inoculating faba bean as compared to the positive control. As the effects of isolates are compared based on shoot dry mass of inoculated faba bean DU1 is the most effective as compared with the remaining isolates and positive control and negative control. When the effects of isolates are compared based on root biomass of inoculated faba bean DU1 is the most effective to increase root biomass of inoculated faba bean as compared with other isolates and positive control and negative control. Meanwhile, DE2 is the least effective when compared with the other isolates. When isolates are compared based on the root dry mass of inoculated faba bean DU1 was observed to be the most effective as compared with the remaining isolates and positive and negative control with respect to root dry mass of faba bean As statistically revealed p=0.002), it has been observed that there is the significant difference among the isolates, concerning root dry mass of inoculated faba bean.

Table 11: Effect of PSB isolates on different growth parameters in Dosha faba bean under greenhouse conditions after 60 days.

	Treatment with isolates								
Parameter	**DU1**	**DU3**	**DU4**	**DU5**	**DE1**	**DE2**	**+Ve control**	**-ve control**	**P≤0.05**
Shoot Length (cm)	58 ±10.58	48± 6.24	50.33± 0.57	71.66±15.27	54.66±5.03	54.3± 3.5	50±1	44±3.6	0.012
Root Length (cm)	12±1	13.33± 1.52	9±1	12.66±3.78	11.33±2.51	10.66 ±1.5	12.66± 1.5	8.66±0.577	0.126

LEAF Number	31.33±3.78	33±1	30.3±2.51	39±4.35	38±2	39.33	36.33±2.54	34±2	0.03
Node Number	13.3±0.57	13.3±2.08	11.66±1.15	13.3±0.57	13±1	12.6±0.57	12.3±0.57	11±1	0.117
Flower Number	7.3±2.3	6±1	4.6±1.52	7±0.00	5.3±0.57	6±1	4.66±0.57	4.66±0.57	0.059
Shoot Biomass	28.7±8.86	17.9±10.32	15.1±6.2	21±6.4	18.8±4.8	17.7±5.08	20.1±5.12	14±6.9	0.363
Shoot dry mass	3.1467±1.1	2.1±1.4	1.63±0.57	2.48±1.03	1.8567±0.34	1.96±0.16	2.9±0.5	1.3433±1	0.267
Root Biomass	7.7±1.13	4.6±3.04	5.08±1.92	4.2±1.5	4.1±2.42	3.8±063	6.7±2.17	2.2±0.9	0.057
Root dry Mass	0.903±0.13	0.46±0.26	0.88±0.09	0.53±0.21	0.42±0.202	0.39±0.94	0.57±0.19	0.19±0.09	0.002

3.6.3. The correlation of Dosha faba bean after 60 day

As shown in the Table below the Pearson correlation of dosha faba bean in similar parameters such as shoot length with shoot length has a strong positive correlation. Node number with shoot length (0.417) *, and pode number with root length (0.510*) have moderately correlatedFlower number with shoot length (0.575**) and flower number with node number0.592** have strongly correlated. However, shoot dry mass is negatively correlated with leaf and node number the pot inoculated with these PSB isolates had a considerable effect on shoot length, root length, leaf number, node number, flower number, shoot biomass and shoot dry mass, root biomass, root dry mass of Dosha *faba bean*.

Table 12: Pearson correlation for Dosha faba bean after 60 day

	Shoot length	Root length	Leaf NO-	- Nodeno	Flower no	Shoot biomass	Shoot dry mass	Root biomass	Root dry mass
Shoot length(cm)	1								
Root length(cm)	0.54	1							
Leaf number	0.203	0.271	1						

Node number	0.417*	0.510*	0.176	1					
Flower the pearson correlation number	0.575**	0.202	0.079	0.592**	1				
Shoot biomass	0.373	0.082	0.163	0.239	0.493*	1			
Shoot dry mass	0.044	0.348	-0.08	-0.124	0.103	0.339	1		
Root biomass	0.053	0.44	0.127	0.447*	0.095	0.460*	0.264	1	
Root dry mass	0.111	0.207	-0.31	0.329	0.163	0.241	0.127	0.578**	1

*Correlation is significant at the 0.05 level (2- tailed)

**Correlation is significant at the 0.01 level (2- tailed)

Node number with,shoot length(0.417), and pode number with root length (0.510*) have moderately correlated

Flower number with shoot length (0.575**) and flower number with node number have strongly correlated

3.7. The effect of bacterial isolates inoculation on Eliodoro Wheat variety growth

3.7.1. The effect of bacterial isolates inoculation on Eliodoro Wheat variety growth after 45 days

When the effect of the isolates is compared based on their shoot length of the inoculated *eliodoro wheat variety*, isolate DU1 was observed to be the most effective in enhancing plant growth as compared with the remaining isolates and the negative control. As statistically revealed (P=0.019), it has been observed that there is a significant difference among the isolates to enhance the growth of *wheat variety* with respect to shoot length. Meanwhile, DE1 is the least effective when compared with other isolates and negative control; as compared to the effect of isolates based on the root length of the inoculated wheat variety, the isolate DU5 is effective. However, as statically revealed p=0.09.it has been observed that there is no significant difference among the isolates and negative and positive control with respect to root length. When comparing the effect of isolates based on the leaf number of inoculated wheat and as revealed p=0.077, it has been observed that there is no significant difference among the isolates and negative control with respect to the leaf number of wheat.

The effects of isolates are compared based on the biomass of inoculated wheat and . All isolates have similar effects. As statically revealed p= 0.139 it has been observed that there is no significant difference among the isolates, negative and positive control. As compared effects of isolates based

on dry mass of inoculated wheat statistically revealed p= 0.73, there is no significant difference observed among the isolates and negative controls.

Table 13: Effect of PSB isolates on different growth parameters in Alidoro Wheat variety under greenhouse conditions after 45 day.

Correlation of wheat after 45-day inoculation

Parameter	**Treatment with isolates**								**P**
	DU1	**DU3**	**DU4**	**DU5**	**DE1**	**DE2**	**DAP**	**CONTROL**	
Shoot length	30.3±1.52	25±5.5	28.6±1.52	29.6±2.5	22.33±2.08	26.33±2.08	30±1	27.3±1.52	0.019
Root length	10.3±2.08	10.83±1.6	12.8±2	12.6±1.5	11.5±0.5	10.5±0.5	8.5±2.1	11.8±1.8	0.09
Leaf number	5.66±0.57	5±1	5±1	5±1	5±1	4.33±0.57	5±1	4.6±0.57	0.77
Biomass	5.03±0.86	4.37±1.8	5.47±1.5	4.6±0.7	3±0.98	3.5±0.3	2.9±0.44	4.5±1.6	0.139
Dry mass	0.86±0.15	0.79±0.32	0.79±0.21	0.79±0.13	0.54±0.201	0.84±0.28	0.75±0.13	0.68±0.15	0.73

Table 14:Pearson correlation after 45 day of wheat

	Shoot length	**Root length**	**leaf number**	**biomass**	**dry mass**
Shoot length	1				
Root lenth	-0.071	1			
Leaf number	-0.04	0.073	1		
Biomass	0.293	0.358	0.205	1	
Drymass	0.212	-0.152	-0.34	0.255	1

3.7.2. The effect of bacterial isolates inoculation on Elioidoro Wheat variety growth after 60 days

When the effect of the isolates is compared based on their shoot length of the inoculated *Eliodoro wheat variety*, isolate DU5 was observed to be the most effective in enhancing the plant growth as compared with the remaining isolates and the negative control, as shown in Table 14. The effect of isolates was compared based on the root length of the inoculated wheat variety.DU 5 is also the most effective as compared with the remaining isolates. As statistically revealed p= 0.02, it has been observed that there is a significant difference among the is isolates with negative control and positive control. With respect to root length, the isolates DU3 and DE1 were the least effective as compared with other isolates. As compared, the effect of isolates based on the leaf number of the inoculated wheat variety statistically revealed p=0.9 it has been observed that there is no significant difference between the isolates and negative controls. When the effect of the isolates is compared based on their biomass and dry mass of the inoculated wheat variety, stastically revealed p=0.06 and 0.063 these have been observed that there is no significant difference among the isolates and the control group with respect to biomass and dry mass of inoculated wheat variety.

Table 15: effects of PSB isolates on different growth parameters in eliodoro wheat variety after 60 day under greenhouse conditions (Values re given as means ± SD for triplicate samples).

Parameter	DU1	DU3	DU4	DU5	DE1	DE2	+ve control	-ve control	P - value
Shoot length in(cm)	66±5.2	61.6±3.21	66.3±9.07	71.66±2.5	68±2	69.6±0.57	68.3±4.7	58.6±8.09	0.121
Root length(cm)	8±1.3	6.1±1.04	7±2.64	9.5±1.8	6.1±0.28	8.83±0.28	9.6±1.52	5.6±1.52	0.02
Leaf number	6.3±0.53	6±1	5.6±0.57	6±1	6±1	6±1	6.3±0.57	5.3±0.57	0.9
Biomass (g)	5.7±1.3	4.43±1.24	5.13±1	6.4±1.34	3.9±0.85	6.13±1.03	5.3±0.7	5±1.21	0.06
Dry mass in(g)	2.24±0.6	1.98±0.2	1.73±0.3	2.27±0.11	1.39±0.1	1.65±0.38	1.67±0.57	1.4±0.19	0.063

3.7.3. The correlation on effects of isolates on wheat growth after 60 day

As shown in the Table below, the effects of isolates based on the correlation of root length with shoot length = (0.430*) have moderately correlated with each other. In contrast, biomass with root length has a negative correlation. The effect of isolates based on biomass with leaf number also has a negative correlation, whereas dry mass with nodenumber has positive correlation.

Table16: Pearson correlation on effects of isolates on wheat growth after 60 day

	Shoot length	**Root length**	**Leaf number**	**Biomass**	**Drymas**
Shoot length	1				
Root length	0.430*	1			
Leaf number	0.161	0.046	1		
Biomass	-0.021	0.268	-0.025	1	
Drymas	0.123	0.276	0.027	0.473*	1

* Correlation is significant at the 0.05 level (2-tailed).

As shown the Table above root length and shoot length have medium correlation and they are significant at 0.05 level. In addition to this root length and shoot length,biomass and dry mass also have medium correlation(0.473*)

Figure 7: effects of psb on eliodo wheat variety

4. DISCUSSION

As stated in the objective, this study aimed to isolate and characterize phosphate solubilizing bacteria from soil samples collected from Dupti Kebele of the Afar region and Deskua Kebele of the Amhara regional state in south Gondar Zone. Soil Physico-chemical analysis revealed that soil samples from study sites had significantly different pH, electrical conductivity, cation exchange capacity, organic matter, and macro-elements (phosphorus, calcium, magnesium, potassium, and nitrogen). From this, a total of 10 phosphate-solubilizing bacteria isolates were isolated from the two study sites, which have distinct ecologies.

Morphological characterization, gram reaction, and biochemical test results showed that all isolates are Gram-positive, white, circular-shaped, and transparent except DU1 and DU5, which are Gram-negative, white, and yellow, circular-shaped bacterial isolates. As a morphological characterization, gram reaction, and biochemical test result revealed DU2, DU3, DU4 , DU6 , DE1, DE2 DE3, DE4 were Gram-positive, white, smooth, circular, rode, transparent, and spore former. When these isolates are characterized biochemically, they all are positive for the catalase test and starch hydrolysis test. However, they are all negative for the indole test and oxidase test. Except for DU3, DU4, and DE1 all these Gram-positive bacteria were negative for the methyl red test.

Based on these observed results and characteristics, these Gram-positive bacteria can be general bacillus. In contrast, DU1 and DU5 isolates are Gram-negative smooth and short rod .As biochemical characteristics revealed, both of these gram-negative bacteria were positive for citrate, motility, glucose, catalase, starch hydrolysis, sucrose, and lactose fermentation tests. However, they are negative for the indole test, methyl red test, Voges Proskauer test. Based on this characterization, these two bacterial isolates can be general Pseudomonas. This finding is supported by (Sun Dram, 1994; Haile,.1999), who they reported that the most efficient and frequently encountered phosphate-solubilizing bacteria belong to the genus Pseudomonas or the genus Bacillus.

All these isolates are grown in acidic medium to alkaline medium between pH6 to pH9 except *DE3, DE4, and DU6, which these were grown in pH6, pH7, and pH8.* All isolates were grown on PVK medium containing 5% and 10% of NaCl. However, only six isolates, four isolates (DU1, DU3, DU4, and Du5) from the Dupti kebele soil sample, two isolates (DE1 and DE2) from Deskua kebele soil sample were effectively grown in 15% and 20% of NaCl. Therefore, these isolates can survive at high concentrations of NaCl (up to 20%). *Indeed*, this result is in line with the previous findings (Uma Maheswar and Sathiyavani, 2012; Chaiharn *et al*., 2009), which reported that.

These isolates also formed clear zones by solubilizing suspended TCP due to the release of organic acids into the surrounding medium. The isolate PSB DU5 (6 mm), DE1 (6 mm), DE2 (6mm) showed maximum solubilization index, followed by PSB DU1 (5.6mm, DU4 (5mm), DU3 (4.5) as shown in Table 7 this finding is in line with (Gaur,1990) which he reported that when PSB microorganisms grown in calcium triphosphate containing medium produce hole zone around their colonies through the production of organic acid.. All isolates were confirmed for their Phosphorus solubilization ability by PVK broth medium for quantitative determination of available phosphorous. This indicated that the isolates PSB DE1, DU5, and DE2 solubilized significantly higher phosphate levels than all other bacterial isolates. An inverse relationship was also observed between the pH value of the culture medium and phosphate solubilization due to organic acid secretion. As shown in Table 9, the pH of the medium was initially neutral (pH=7). However, as the time of incubation increased, the pH of PVK broth media decreased from the initial pH after inoculation with the isolates. Based on the observation, the pH of the media inoculated with isolate

DU2=3.5, DE2 =3.72 DU1=3.8, DU3=3.8, DU4=3.87, and DE3=3.9 DU5=4, DE1=4 were recorded after fifteen days of incubation. Therefore, the pH of all bacterial inoculated PVK medium decreased from pH 7 to PH3.5,pH3.8 and pH3.9 PH4 were recorded. This observed result is agreed with (Rodr Guez and Fraga,1999; Chen *et al*., 2006; Ivanova et al., 2006; Muleta *et al*., 2013; Vyas and Gulati, 2009; Gaur, 1990) stated that phosphate solubilizing microorganisms formed clear zones by solubilizing suspended TCP due to the release of organic acids into the surrounding medium. This leads to increased P availability, which ultimately increases plant P uptake.

In the present study, 10 PSB strains were isolated, and six of these were efficient PSB isolates (DU1, DU3, DU4, DU5, DE1, and DE2 solubilized inorganic phosphate with solubilization index ranged from 4.5 to 6. They were selected for further studies for pot experiments of the Dosha faba bean and Alidoro wheat variety. The present study was conducted to screen the potential Phosphate solubilization and its use as a biofertilizer.DU1, DU3, DU4, DU5, DE1, and DE2 were selected for pot scale trial because of their positive, their efficient phosphate solubilization ability on plate assay as well as their release of free P in liquid culture medium and their abilities to enhance plant growth

This study showed the isolated PSB had a considerable effect on agronomic properties such as plant height, number of leaves, number of nodes, number of flowers, biomass of shoot, dry mass of shoots, biomass of root, and dry mass of root. Isolate PSB DU5 showed the best effect on shoot length (71.66 ±15.27), root length(12.66 ±3.7), leaf number (39±4.35), nod number(13.3±0.57). Isolate PSB DU1 and PSB DE1, DE2 showed second and third-best effects on the shoot length of Dosha faba bean growth, respectively. Similarly, PSB DU3 significantly increased the root length (13.33±1.52) of faba bean. DU1, DU3, DU5, and DE1 had a similar effect on pod number of f*aba* bean. All PSB isolates significantly increased the shoot length, fresh shoot biomass, and root length of faba bean as compared with positive control and negative control of the faba bean pot experiment. This result is agree (Dey *et al*., 2004; Fernández *et al*.,2007; Vikram and Hamzehzarghani, 2008; Hariprasad andNiranjana, 2009; Yu *et al*., 2011). They reported that the increase in shoot length, root length, leaf number, shoot dry weight, and root dry weight of bean plants inoculated with PSB strains could be attributed to greater absorption of nutrients, especially Phosphorus uptake. The result of analyses of variances showed that most of these PSB isolates significantly ($p \leq 0.05$) increased all the investigated parameters such as plant height, fresh/wet weight (biomass), dry weight, number of leaves, and flowering as compared with positive and negative control.

The correlation analysis revealed that plant shoot length and plant root length were found to be positively correlated with the number of leaves and strongly correlated with the number of nodes. Other studies showed that plant height and shoot (number of leaves) are usually positively correlated.

Conclusion and recommendation

The present study ten phosphate solubilizing bacterial isolates coded as DU1, DU2, DU3, DU4, DU5, DU6, DE1, DE2, DE3, DE4 were isolated and biochemically characterized. All isolates were found to be efficient in the solubilization of tricalcium phosphate. However, only six isolates were used for the pot experiment due to their maximum phosphate solubilization and stress tolerance ability.

Conflict of interest

The author did not declare any conflict of interest.

Authors contribution

BD was involved in designing the study, data collection, writing the manuscript, and data analysis. TS and KGM were involved in designing the study, data collection, and writing of the manuscript. MI and PB were involved in the review and editing of the manuscript. NB was involved in the review and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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