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Research Article

Multi-trait plant probiotic bacteria improved growth and nutrient uptake of potato (*Solanum tuberosum* L.) varieties in Eastern Ethiopia

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Abstract

Mobilizing multi-functional plant probiotic bacteria in the rhizosphere plays a significant role in improving soil fertility and sustainable agricultural production and productivity. This study assessed the effect of selected plant probiotic bacteria (PPB) on the growth and nutrient uptake of three potato varieties (Bete, Gudene, and Bubu) under greenhouse condition. The experiment was arranged in a completely randomized design with seven species of probiotic bacteria: Providencia rettgeri, Enterobacter aerogenes(Kleb.mobilis), Raoultella planticola/ornithicola, Lysinibacillus sphaericus, Serratia marcescens ss marcescens, Lysinibacilus fusiformis, and Bacillus Flexus. and four replicates. The findings revealed that inoculated plant probiotic bacteria improved the surrogate agronomic character of potato varieties by 20-40%, with Providencia rettgeri and Lysinibacillus fusiformis demonstrating best performance. Following innoculation of plant probiotic bacteria, the Bete variety showed to have better number of tubers (TN) and root growth (RDW) than the Bubu and Gudene varieties. Inoculation of potato varieties with Lysinibacillus fusiformis improved average plant and tuber biomass by 30%, followed by Providencia rettgeri(20%). All isolates significantly increased Bete's tuber N, P, and Fe nutrient content by 76.17%, 72.59%, and 65.44%, respectively. Inoculation of potato varieties with Lysinibacillus fusiformis and Raoultella planticola/ornithinolytica improved nutrient uptake of potato shoots and tuber by 60% and the Bete variety responding better to inoculation.The study also found that shoot dry weight, tuber dry weight, dry matter content, and root dry weight positively correlate with plant growth promotion characteristics. Generally, this study evidenced that the PPB isolates have the potential as bio-inoculants for potato varieties. However, the isolates have to be further testedunder field conditions for subsequent evalutation.

Keywords: *Enterobacter aerogenes* (Kleb. Mobilis), growth-promoting trait, *Lysinibacillus sphaericus*, plant probiotics, *Serratia marcescens*

Introduction

Potato (*Solanum tuberosum* L.) is a starchy root vegetable native to the Americas that is consumed as a staple food in many parts of the world. According to the FAO data, a total of 376 million tonnes of potatoes were produced worldwide, of which China (94 million tonnes) and India (54 million tonnes) are the largest potato-producing countries [1]. The crop is grown in more than 100, mainly in temperate and tropical countries such as Egypt, Ethiopia, Sudan, and Kenya highlands [2]. It is widely cultivated at altitudes of approximately 1500–4200 meters and temperatures of 18–20°C on a deep, well-drained, friable soil with a pH range of 5 to 6.5.[3] In Ethiopia, it is domesticated over a century and two decades, from a garden crop in a few regions to a staple crop produced in many regions under different agro ecological conditions [4].It ranks first in the category of root and tuber crops (CRP) in terms of area coverage (60%) and total production (51%) with potato production of 1,044,436.359 tons (76,677.64 ha), with a yield of 13.62 tons/ha^{-1}[4].

Vegetable production in Ethiopia is operated under different challenges, which results in low yield [5]. Moreover, conventional agricultural practices frequently rely on synthetic fertilizers and pesticides which have adverse effects on humans, animals, and the environment [6]. Considering all the harmful effects of synthetic chemical fertilizers remind the need develop a sustainable and eco-friendly alternative like the use of Plant Growth Promoting Rhizobacteria (PGPR). Plant probiotics are the use of PGPR to enhance the growth and development of the host plants when applied in adequate amounts [7]. They are well known to enhance plant growth and improve yield by increasing plant nutrient use efficiency through nutrient solubilization and mineralization [8,9] such as organic acid production [10], N-fixation, Siderophore activity [11]and synthesis of phytohormone [12] .

Similarly, PPB enhance plant growth and development through mineralization, and nutrient uptake for their hosts and the presence of direct antagonistic traits on pathogens [13]. Thus, the application of plant-associated probiotic bacteria could be a potentail alternatives to agrochemicals, leading to more resilient and productive agricultural systems [14]. By isolating and characterizing these beneficial bacteria, researchers can identify the most effective strain for enhancing vegetable growth and resilience. Andreas Oswald and Pamela Calvo Valez [15] collected a total of 346 strains of 5 bacterial genera from the rhizosphere of potato plants collected in different regions of the Central Andes. Results of field trials with potatoes were variable sometimes increasing yields by up to 60% but also showing no response in some cases necessitating the need for screening specific probiotic bacteria for different varieties of potato plants.

For the last few decades, there has been a lot of interest in the study of PGPR as bio stimulants, bio fertilizers, and bio pesticides for different vegetable plants such as tomatoes [16,17] pepper, and others [18] in Ethiopia. Several studies also showed that the association of plant probiotic bacteria with potato rhizosphere in Ethiopia inhibits different phytopathogens of potatoes and improves production [19–21]. Many of the hitherto studies focused on the association of biocontrol potential for selected pathogens and agro ecological areas. Another study by Guchi [22] emphasized the need for integrated production management of potatoes by focusing on biocontrol methods together with the use of resistant varieties, intercropping, use of certified disease-free seed, use of selective fungicides, and cultural practices.

As a result, the issue of PPB interaction with potatoes in particular and food crops in general remains a critical matter for agriculture Thus, this study was designed to evaluate potential plant probiotic bacteria on potato production under greenhouse conditions.

Materials and Methods

Description of the study site

The research was executed at the Rare research field of Haramaya University. Haramaya University is situated in the eastern region of Ethiopia, and it is 25 kilometers away from North West of Harar town. It is located at an elevation of 2006 meters above sea level, with a latitude of 9°24"53' N and a longitude of 42°2"9' E. The location is typical of a sub-humid, mid-altitude agro climatic zone and features a bimodal rainfall distribution pattern [23]. The experimental soil for the pot trial had a loamy sand texture with a low organic carbon content of 0.9% and a composition of 10% clay, 82% sand, and 8% silt. Based on the phosphorus (P) rating (m/kg) by Wogi et al [24], the soil at the experimental site has a low accessible P concentration (<3). The soil's pH of 6.96 falls within the pH preference range of 5–7 for the growth of potatoes [25], while the soil's total nitrogen of 0.05% is classified medium as suggested by wogi et al. [24].

The experimental materials, treatments, and design

Three varieties named *Bubu* (CIP-384321.3), *Gudene* (CIP-386423.13), and *Bete* (farmer's cultivar) were obtained from the Potato Improvement Program of Haramaya University. Potato varieties were selected based on tuber seed availability and farmer's preferences for tuber yield and quality.

***Table 1**: Description of plant probiotic bacteria isolates as evaluated and characterized at Haramaya University and used for evaluation on potato cultivars under Greenhouse experiment*

S.No	PPB	Plant growth-promoting properties						
		Siderophore	Ps	Zns	N fixation	Organic acid production	GA production	IAA production
1	HK1	++	++	++	+	+	++	++
2	AR2	++	++	++	+	+	++	++
3	BSO5	++	++	+	+	+	+	++
4	SBO4	++	++	++	+	+	+	++
5	BSO1	++	++	+	+	+	+	++
6	SLL1	++	++	++	+	+	++	++
7	STG5	++	++	+	+	+	+	++

PPB = Plant Probiotic Bacteria, Ps = Phosphorus solubilization, Zns = Zinc solubilization, N= Nitrogen fixation, GA = Gibberellic acid and IAA = Indole Acetic Acid

A total of promising 25 PPB isolates were screened from potato rhizosphere. Seven potential PPB isolates; SBO4 (*Lysinibacillus sphaericus*), HK1 (*Providencia rettgeri*), STG5 (*Bacillus flexus*), AR2 (*Enterobacter aerogenes; Kleb. Mobilis*), SLL1 (*Lysinibacillus fusiformis*), BSO5 (*Raoultella planticola/ornithinolytica*), and BSO1 (*Serratia marcescens ss marcescens*) were selected from 25 PPB isolates based on their plant growth promoting properties (Table 1). The greenhouse experiment consists of 24 treatments viz. seven potential PPB with negative control (non-inoculated tubers) and three potato varieties in factorial combination. The experiment was laid out in a completely randomized design (CRD) with four replications.

Experimental procedures and management

Potato seed tuber surface sterilization and bacterial inoculum preparation

Healthy medium-sized sprouted tubers of *Bubu, Gudene* and *Bete* varieties were used and washed with water. Then, each tuber was surface sterilized with 70% alcohol for three minutes, followed by shaking with 1% hypochlorite for five minutes, and rinsed five times with sterile distilled water and allowed to dry as described somewhere else [26].

In vivo plant growth promotion assay

In the greenhouse experiment, a pot trial was conducted to evaluate the effect of multi-traits PPB isolates. The PPB inoculum was prepared from culture grown in nutrient broth and incubated at 28±2 ^{o}C for 48 hrs. A uniform cell density (10^8 CFU mL^{-1}) of each PPB suspension was maintained at OD600 =0.6. Each sprouted tuber of potato varieties was coated with 20% sugar solution as an adhesive before planting the tubers in pots. Then after, rolled into the suspension of each PPB adjusted at 10^8 CFU mL^{-1} until uniformly coated for the inoculation of tubers.

Planting and growing of potatoes

The undisturbed soil cores were collected from cereal crop planted farm after harvest in a zig-zag pattern using augur. Once taken, soil cores were crushed and passed through a 2 mm sieve to have a uniform particle size. The pots with 29 cm height and the upper 28 and 20 cm bottom diameter, respectively, were filled with 10 kg of soil. The tubers of three potato varieties were treated with a suspension of PPB isolates and non-treated tubers (control) planted in each pot at a 5 cm depth labeled with four replicates and placed on working beds randomly. Distilled water was applied to each pot at field capacity. Fifteen days later, a second bacterial inoculation was performed, in which 20 mL of the PPB inoculums (10^8 CFU mL^{-1}) was added per pot using a sterile syringe. Pots were irrigated every four days until plant emergence and then every seven days until maturity. The pots were kept free of weeds by hand weeding without using herbicides.

The haulms were cut two weeks before collecting tubers from pots when the plants reached physiological maturity or haulms turned yellow color. The tubers were collected carefully from each pot and data were collected.

Data collection

Potato tuber yield and yield-related traits

The data for plant height, main stem thickness, number of leaves, internode length, and leaf area index were taken/collected at the flowering (or tuber initiation) stage. Plant height was measured from the base to the apex of the plants and internode length was measured using a meter scale. Main stem thickness was measured using a digital caliper. The leaves produced by plants in each pot were counted, and the leaf area index was measured following standard procedures [27]. Shoot fresh and dry weights were measured at the physiological maturity (haulms turned to yellow color) at the time of dehaulming. The aboveground plant parts were collected carefully and measured with sensitive balance after the haulms were cut and oven dried at 72°C until constant weight and the dry weight of shoots was measured.

The number of tubers per pot and tuber fresh weight was registered after all tubers in each pot were collected after 15 days of dehaulming. The average tuber weight was determined by dividing the weight of all tubers obtained from each pot and divided by the total number of tubers. Five tubers of different sizes were randomly taken from each treatment, chopped into small 1-2 cm cubes, weighted and oven-dried at 72°C for 72 hrs and dried. Each sub-sample was weighed and recorded as dry weight. The total dry matter content (DMC) was calculated based on the study described somewhere else [28]. Total starch content (g/100g) was estimated from dry matter as starch content (%) = 17.55 + 0.891 * (tuber dry weight (%) – 24.182) as described in other study [29]. The tuber dry weight was estimated as a multiple of tuber dry matter content and tuber fresh weight.

Potato shoot and tuber nutrient contents

The macro- and micronutrients : Fe, Zn, P, and N were determined following standard procedures [28]. After being oven-dried for 48 hrs at 95°C, the shoots and tuber were ground. Then, the N concentration was determined using full digestion in concentrated H_2SO_4 and subsequent distillation using the micro-Kjeldahl method. A metavanadate colorimetry method was used for P determination and the absorbance was measured using UV-VIS spectrophotometer. A flame-atomic absorption spectrometer was used to measure the contents of Fe and Zn [30].

Data analysis

All collected data were subjected to analysis of variance (ANOVA) using the Gene Stat software version 18. The Least Significant Difference (LSD) test at the 5% level of probability was used for mean comparison following the significant differences results from the ANOVA. Pearson correlation analysis was used to evaluate the relationship between growth-related and yield-related parameters and the nutrient uptake parameters. In the greenhouse experiment, the percent of increment was calculated to evaluate the difference between the control and treatments using the formula:

$$\frac{Treatment - control}{Control} \times 100$$ ---------- **Equation.**

Results

Growth promotion effect of plant probiotic bacterial isolates inoculation on potato agronomic traits

Analysis of variance on growth promotion effect of PPB on potato varieties revealed that traits like plant height, stem thickness, internode length, leaf area, leaf area index, shoot weight, Root fresh weight, and tuber fresh weight were statically and significantly influenced by both treatment and the interaction effect (Table 2).

The effect of both treatment and variety was found highly significant ($p < 0.001$) on studied parameters such as plant height, leaf number, shoot fresh weight, tuber fresh weight, and root fresh weight. Internode length and leaf area index were affected significantly ($p < 0.01$) by treatment and steam thickness by variety.

***Table 2**: Mean square of treatment, variety, and their interaction of plant probiotic bacteria on potato growth-related parameter*

Source of variation	DF	Potato Growth Related Parameter							
		PLH	ST	INL	LAI	LN	SFW	TFW	RFW
Rep	3	27.25^{NS}	1.1253^{NS}	0.0325^{NS}	0.001026^{NS}	718.6^{NS}	60.01^{NS}	350.9^{NS}	2.52^{NS}
Var	2	738.22^{***}	7.5333^{**}	0.0545^{NS}	0.089557^{***}	123011.1^{***}	1477.29^{***}	12809.3^{***}	285.571^{***}
Trt	7	238.93^{***}	5.9256^{***}	0.8111^{**}	0.01779^{**}	27579.8^{***}	1947.44^{***}	7183.2^{***}	120.333^{***}
Var.Trt	14	114.95^{***}	1.7843^{*}	0.623^{**}	0.004838^{NS}	10546.7^{***}	697.02^{***}	2267.8^{***}	52.24^{***}
Error	69	33.98	0.8806	0.2567	0.005835	270.7	50.37	272.8	3.371

Note: *, **, ***: statistically significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ probability level respectively; NS: Not Significant. PLH: plant height, LN: leaf number, SFW: shoot fresh weight, TFW: tuber fresh weight, RFW: root fresh weight. INL: internode length and LAI: leaf area index

The interaction effect of variety and treatment on growth-related parameters were highly significant ($p < 0.001$) on plant height, leaf tuber, shoot fresh weight tuber fresh weight, and root fresh weight. Moreover, the interaction effect of variety and treatment showed significant difference on internode length and steam thickness at $p<0.01$) and $p < 0.05$, respectively. However, the interaction effect of variety and treatment did not significantly affect the leaf area index of the potato.

The plant height of the *Bete* variety was significantly ($p<0.001$) increased by inoculation of BSO1 (*Serratia marcescens ss marcescens*), BSO5 (*Raoultella planticola/ornithinolytica*), and AR2 (*Enterobacter aerogenes; Kleb. Mobilis*). The longest PLH (67.83cm) was observed on *Bete* inoculated with BSO1 (*Serratia marcescens ss marcescens*), and the shortest PLH (44.32 cm) was observed on uninoculated pots (Table 3). Similarly, the *Bete* variety inoculated with AR2 (*Enterobacter aerogenes; Kleb. Mobilis*) and SBO4 (*Lysinibacillus sphaericus*) significantly ($p<0.001$) increased leaf number compared with control. The interaction effect of variety and treatment on leaf number revealed that variety *Bubu* inoculated with HK1 (*Providencia rettgeri*) did not exhibit a significant difference compared with the control. The internode length was significantly ($p < 0.01$) affected by the inoculation of STG5 (*Bacillus flexus*) on variety *Bubu* par with AR2 (*Enterobacter aerogenes; Kleb. Mobilis*) on the *Bete* variety. The smallest shoot fresh weight (85.8 g) was observed on the uninoculated *Bubu* and *Bete* variety whereas the highest shoot fresh weight (143.2 g) was recorded by *Bete* inoculated with HK1 (*Providencia rettgeri*) which exceeds 32.6 % over the control.

Bacterial inoculation significantly increased many of the agronomic character compared to uninoculated control plants, except LAI on all varieties and PLH, INL, and RFW on one of the variety. Interestingly, the *Bubu* variety did not show good performance on parameters such as INL, ST, SFW, and RFW under different inoculants. Most of the inoculants did not show significant differences in TFW and INL on *Bete* and *Gudene* varieties.

The innoculants showed to cause variation of agronomic characters across the potato varieties (Table 3). Consequently, the SLL1 (*Lysinibacillus fusiformis*) improved the agronomic character of the *Bubu* and *Gudene* varieties followed by HK1 (*Providencia rettgeri*) on the *Bete* variety. The findings of the study indicated that some inoculants improved different agronomic characters with a range of 20–40% as compared to the uninoculated control. In the study inoculants like HK1 (*Providencia rettgeri*) and SLL1 (*Lysinibacillus fusiformis*) showed best performance in improving the agronomic characters in all varieties followed by inoculant AR2 (*Enterobacter aerogenes; Kleb. Mobilis*), and STG5 *(Bacillus flexus).*

***Table 3**: Mean treatment and variety of potato growth-related parameter*

Trt x VAR	Potato Growth Related Parameter							
	ST(mm)	PLH(cm)	INL(cm)	LN	LAI(cm^2)	SFW(g)	TFW(g)	RFW(g)
BTxCONT	18.35^{i}	44.32^{gh}	3.567^{f}	307.8^{j}	1.24^{bc}	85.8^{j}	253.9^{k}	12.7^{j}
BTxHK1	21.52^{ab}	56.77^{bcd}	4.642^{abc}	509.8^{bc}	1.2175^{bc}	143.2^{a}	331.3^{bcd}	27.6^{a}
BTxAR2	20.87^{abcde}	61.37^{ab}	4.967^{ab}	564.8^{a}	1.25^{abc}	134^{ab}	278.7^{j}	18.5^{gh}
BTxBSO5	21.65^{a}	67.25^{a}	4.083^{cdef}	482^{d}	1.2975^{abc}	126.5^{bc}	318.9^{defg}	22.57^{cde}
BTxSBO4	20.34^{abcdef}	48.00^{fgh}	3.992^{cdef}	554.8^{a}	1.36^{abc}	106.5^{efg}	332^{bcd}	13.57^{j}
BTxBSO1	20.1^{cdefg}	67.83^{a}	4.583^{abcd}	472^{d}	1.285^{abc}	111.6^{def}	338.2^{bcd}	$15.02i^{j}$
BTxSLL1	20.82^{abcde}	52.47^{cdefg}	3.817^{ef}	508.2^{bc}	1.3175^{abc}	108.7^{ef}	346.4^{bc}	18.67^{fgh}
BTxSTG5	21^{abcd}	56.78^{bcd}	4.083^{cdef}	489.8^{cd}	1.265^{abc}	105.5^{efg}	282.5^{ij}	16.57^{hi}
BUxCONT	18.31^{i}	43.37^{h}	3.567^{f}	302.8^{j}	1.32^{abc}	85.8^{j}	252.6^{k}	13.57^{j}
BUxHK1	19.78^{defgh}	44.32^{gh}	4.375^{bcde}	307.8^{j}	1.38^{abc}	96.7^{ghi}	253.9^{k}	15.05^{ij}
BUxAR2	19.75^{defgh}	46.65^{fgh}	4.333^{bcde}	381^{gh}	1.3925^{abc}	103.9^{fgh}	329.2^{bcde}	24.2^{bc}
BUxBSO5	19.78^{defgh}	46.63^{fgh}	3.875^{def}	366.5^{hi}	1.34^{abc}	87.2^{ij}	285.4^{hij}	14.8^{ij}
BUxSBO4	20.3^{bcdef}	48.61^{defgh}	4.208^{cdef}	355^{i}	1.38^{abc}	98^{gh}	314.9^{defg}	20.25^{efg}
BUxBSO1	21.32^{abc}	46.66^{fgh}	4.042^{cdef}	418^{ef}	1.35^{abc}	95^{hij}	307.6^{efgh}	17.07^{hi}
BUxSLL1	18.84^{ghi}	53.57^{bcdef}	4.417^{bcde}	408.2^{ef}	1.365^{abc}	137.8^{a}	284.2^{j}	$21.1^{de}f$
BUxSTG5	19.35^{fghi}	48.14^{efgh}	5.292^{a}	367^{hi}	1.345^{abc}	114.7^{de}	302.8^{fghi}	21.92^{cde}
GUxCONT	18.56^{hi}	44.32^{gh}	3.817^{ef}	306.2^{j}	1.2425^{bc}	87.2^{ij}	278.7^{j}	15.05^{ij}
GUxHK1	19.58^{efghi}	58.30^{bc}	4.25^{cdef}	516.2^{b}	1.3725^{abc}	126.3^{bc}	337.7^{bcd}	27.62^{a}
GUxAR2	19.7^{defgh}	47.39^{fgh}	4.025^{cdef}	395.5^{fg}	1.4275^{ab}	112.5^{def}	$300.7g^{hij}$	26.62^{ab}
GU xBSO5	20.03^{cdefg}	57.96^{bc}	4.375^{bcde}	399.8^{fg}	1.405^{abc}	109^{ef}	325.9^{cdef}	23.2^{cd}
GUxSBO4	19.48^{fghi}	48.74^{defgh}	4.292^{bcde}	406^{ef}	1.4475^{a}	105.8^{efg}	351.7^{b}	21.5^{de}
GUxBSO1	20.84^{abcde}	56.29^{bcde}	4.375^{bcde}	358.8^{hi}	1.375^{abc}	105.2^{efg}	351.6^{b}	23.62^{cd}
GUxSLL1	20.38^{abcdef}	58.06^{bc}	4.067^{cdef}	427^{e}	1.4225^{ab}	133.8^{ab}	380.7^{a}	27.1^{a}
GUxSTG5	19.96^{defg}	46.63^{fgh}	4.25^{cdef}	471^{d}	1.3425^{abc}	121.5^{cd}	323.6^{cdefg}	23.2^{cd}
LSD (5%)	1.324	8.223	0.715	23.21	0.108	10.012	23.3	2.59
CV%	4.7	11.2	12	3.9	5.7	6.4	5.3	9.2

BU: *Bubu;* BT: *Bete*; GU: *Gudene;* PLH: plant Height, LN: leaf number, SFW: shoot fresh weight, TFW: tuber fresh weight: RFW: root fresh weight. INL: internode length and LAI: leaf area index. Different letters across the column indicate significant differences at $p \leq 0.05$ according to the LSD test

Tuber fresh weight was significantly ($p<0.001$) improved by SLL1 (*Lysinibacillus fusiformis*) inoculation. However, the isolate does not show a statistically significant difference over the control inoculated on the *Bubu* variety (Table 3). *Bete* and *Gudene* varieties inoculated with HK1 (*Providencia rettgeri*) and inoculation of SLL1 (*Lysinibacillus fusiformis*) on *Gudene* significantly ($P < 0.001$) increased tuber fresh weight compared with control. *Bubu* inoculated with SLL1 (*Lysinibacillus fusiformis*) and *Bete* varieties inoculated with HK1 (*Providencia rettgeri*) and AR2 (*Enterobacter aerogenes; Kleb. Mobilis*) significantly ($P < 0.05$) increased stem thickness compared to control. Significantly highest stem thickness (143.2 mm), which was increased by 40.08 % over the control, was recorded by *Bete* inoculated with HK1 (*Providencia rettgeri*).

PPB Inoculation Effect on Potato Yield-Related Parameters

Analysis of variance on growth promotion effect of PPB on potato yield-related parameters revealed that traits like shoot dry weight, tuber dry weight, tuber number, and root dry weight were statically and significantly influenced by both treatment and the interaction effect (Table 4).

Table 4*: Mean square of treatment, variety, and their interaction on potato yield-related parameters*

SOV	DF	Potato Yield Related Parameter						
		SDW	TDW	TN	RDW	ATW	DMC%	SC
Rep	3	5.492^{NS}	46.16^{NS}	5.014^{NS}	1.0004^{NS}	27.837^{NS}	1.184^{NS}	0.94^{NS}
Var	2	157.098^{***}	148.36^{*}	63.5***	11.1884***	356.211***	25.004***	19.85***
Trt	7	58.721^{***}	264.2^{***}	17.327***	15.9203***	51.643***	7.901*	6.272*
Var.Trt	14	19.455^{***}	87.1^{**}	5.131***	5.4118***	43.383***	4.745^{NS}	3.767^{NS}
Error	69	2.119	46.83	2.021	0.8748	9.065	2.921	2.319

Note: *, **, ***: statistically significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ probability level respectively; NS: Not Significant; SOV: source of variation; SDW: shoot dry weight; TDW: tuber dry weight; TN: tuber number RDW: root dry weight; ATW: average tuber weight; DMC%: Dry matter content of tuber and SC: starch content (g/100g)

The effect of both treatment and variety was found highly significant ($p< 0.001$) on studied parameters such as shoot dry weight, tuber number, average tuber weight and root dry weight. Tuber dry weight was improved significantly ($p<0.05$) by variety. The interaction effect of variety and treatment on growth-related parameters was highly significant ($P<0.001$) on shoot dry weight, tuber number, average tuber weight and root dry weight. Whereas, the interaction effect of variety and treatment on tuber dry weight was significant ($P<0.01$).

Effect of PPB Inoculation on Potato Varieties Yield Related Parameters

The shoot dry weight of the *Bete* variety was significantly ($p < 0.001$) increased by inoculation of HK1 (*Providencia rettgeri*) and SLL1 (*Lysinibacillus fusiformis*) par with the inoculation of the *Gudene* variety with STG5 *(Bacillus flexus)*. The maximum shoot dry weight (27.0 g) was observed on *Bete* inoculated with SLL1 (*Lysinibacillus fusiformis*), and the minimum shoot dry weight (17.22 g) was observed on the control (Table 5). Similarly, *the Gudene* variety inoculated with SLL1 (*Lysinibacillus fusiformis)* significantly ($P<0.001$) increased the tuber number compared with the control. On the contrary, a significant difference ($P<0.001$) was not found in the interaction between the *Gudene* variety inoculated with BSO1 (*Serratia marcescens ss marcescens*) compared with the control. A significant difference ($P<0.01$) was observed in the interaction between SBO4 (*Lysinibacillus sphaericus*) par with SLL1 (*Lysinibacillus fusiformis*) and *Gudene* variety on Tuber dry weight. However, a significant difference was not observed on *Bubu* variety inoculated with HK1 (*Providencia rettgeri*) as compared to the control.

The result demonstrated that inoculation significantly increased SDW, TDW, TN, ATW and RDW compared to uninoculated control. All isolates significantly increased the SDW of Bete variety with the range of 29-37% compared to the uninoculated control. The isolates like AR2 (*Enterobacter aerogenes; Kleb. Mobilis*), SBO4 (*Lysinibacillus sphaericus*), and SLL1 (*Lysinibacillus fusiformis*) increased SDW by 14, 14 and 33%, respectively (Table 4). More importantly the SLL1 (*Lysinibacillus fusiformis*) increased the SDW of all varieties. The potato varieties showed variation on ATW when inoculated with different PPB isolates (Table 5).

Thus, the Bete variety performed better by producing more tubers (TN) with a mean of 32% (22-67%) over control plants followed by the *Bubu* and *Gudene* varieties, with a mean TN of 22% (17-33%), and mean TN of 17% (16-25%), respectively. The isolates also improved RDW of the varieties from 22%, (*Bubu* variety) to 39% (*Gudene* variety (mean=31%) indicating that root growth was more enhanced by inoculation than TDW (12%), SDW (20%), and (TN (24%). In general, inoculation of potato varieties with SLL1 (*Lysinibacillus fusiformis*) improved average plant and tuber biomass by 30% followed by 20% with HK1 (*Providencia rettgeri*).

***Table 5**: Mean of treatment and variety on potato yield-related parameter*

TrtxVar	Potato Yield Related Parameter						
	SDW	TDW	TN	RDW	ATW	DMC%	SC
BTxCONT	17.22^{g}	65.6h^{i}	8.75^{i}	4.4^{i}	29.94^{b}	25.85^{a}	19.03^{a}
BTxHK1	26.92^{a}	75.57^{bcdefg}	11.25^{gh}	8.25^{abcd}	30.33^{b}	22.91^{ab}	16.42^{ab}
BTxAR2	25.47^{abc}	66.22^{ghi}	14.75^{abc}	8.8^{a}	19.05^{jk}	23.72^{ab}	17.14^{ab}
BTxBSO5	24.25^{bc}	72.17^{cdefghi}	12.25^{defg}	7.4^{bcdef}	26.66^{bcde}	22.60^{ab}	16.14^{ab}
BTxSBO4	24.5^{bc}	79.97^{abc}	12.25^{defg}	4.675^{i}	27.38^{bcd}	24.05^{ab}	17.44^{ab}
BTxBSO1	25.9^{abc}	71.6^{cdefghi}	9.5h^{i}	4.75^{i}	36.26^{a}	21.13^{b}	14.83^{b}
BTxSLL1	27.0^{a}	74.5^{bcdefgh}	12^{efg}	8.625^{ab}	28.98 ^{bc}	21.38^{ab}	15.05^{ab}
BT xSTG5	24.05^{c}	69.17^{defghi}	11.75^{fg}	6.1^{fgh}	24.03^{defgh}	24.52^{ab}	17.85^{ab}
BUxCONT	18.23^{fg}	63.62^{i}	11.25^{gh}	4.675^{i}	23.17^{defghij}	25.18^{ab}	18.44^{ab}
BUxHK1	18.72^{efg}	65.6^{hi}	14.75^{abc}	5.125^{hi}	17.25^{k}	25..85^{a}	19.03^{a}
BUxAR2	20.48^{de}	78.55^{abcd}	13.5^{bcdef}	6.575^{efg}	24.55^{defg}	23.85^{ab}	17.26^{ab}
BUxBSO5	18.68^{efg}	66.92^{fghi}	13.5^{bcdef}	5.45^{ghi}	19.74^{ijk}	23.43^{ab}	16.88^{ab}
BUxSBO4	21.3^{d}	75.9^{bcde}f	15^{abc}	8.025^{abcd}	21.22^{ghijk}	24.10^{ab}	17.48^{ab}
BUxBSO1	19.7^{def}	78.45^{abcd}	14.25^{abcd}	5.025^{hi}	21.05^{ghijk}	25.53^{ab}	18.75^{ab}
BUxSLL1	24.1^{c}	68.55^{efghi}	13.75^{bcdef}	7.15^{cdef}	21.75^{fghij}	24.12^{ab}	17.5^{ab}
BUxSTG5	18.8^{efg}	70.72^{cdefghi}	15.5^{ab}	6.975^{def}	20.98^{ghijk}	23.35^{ab}	16.81^{ab}
GUxCONT	18.22^{fg}	66.22^{ghi}	11.75^{fg}	4.675^{i}	19.80^{hijk}	23.72^{ab}	17.14^{ab}
GUxHK1	24.67^{bc}	75.75^{bcdefg}	15^{abc}	8.4^{abc}	23.71^{defghi}	22.44^{ab}	16^{ab}
GUxAR2	18.23^{fg}	67.62^{fghi}	15.25^{ab}	8.175^{abcd}	22.56^{efghij}	22.50^{ab}	16.05^{ab}
GU xBSO5	21.05^{d}	72.27^{cdefghi}	13.75^{bcdef}	7.75^{abcde}	23.85^{defghi}	22.18^{ab}	15.77^{ab}
GUxSBO4	21.02^{d}	86.35^{a}	13^{cdefg}	6.1^{fgh}	27.22^{bcd}	24.56^{ab}	17.88^{ab}
GUxBSO1	19.73^{def}	77.92^{abcde}	13.75^{bcdef}	7.075^{def}	25.53^{cdef}	22.19^{ab}	15.78^{ab}
GUxSLL1	25.85^{abc}	83.35^{ab}	16^{a}	8.2^{abcd}	23.83^{defghi}	21.89^{ab}	15.51^{ab}
GU xSTG5	26.3^{ab}	71.37^{cdefghi}	14^{abcde}	8.05^{abcd}	23.24^{defghij}	22.04^{ab}	15.64^{ab}
LSD (5%)	2.054	9.654	2.005	1.319	4.247	2.411	2.148
CV%	6.6	9.4	10.8	14	12.4	7.3	9

BU: *Bubu;* BT: *Bete*; GU: *Gudene;* SDW: shoot dry weight; TDW: tuber dry weight; TN: tuber number RDW: root dry weight; ATW: average tuber weight; DMC%: Dry matter content of tuber and SC: starch content (g/100g). Different letters across the column indicate significant differences at $P \leq 0.05$ according to the LSD test.

Other inoculants such as SBO4 (*Lysinibacillus sphaericus*), AR2 (*Enterobacter aerogenes; Kleb. Mobilis*), and STG5 *(Bacillus flexus)* increased these parameters with an average higher than 20% compared to the uninoculated control plants. The *Bete* variety (75%) responded better to inoculation in terms of the significant number of production parameters followed by the *Gudene* (71%) and *Bubu* (64%) variety (Table 5). Furthermore, the findings showed interspecies differences in biomass accumulation where isolate SLL1

(*Lysinibacillus fusiformis*) increased the overall mean biomass accumulation by 31% followed by all others with biomass increase of 16-23% compared to the uninoculated control (Table 5).

Effect of PPB Inoculation on Potato Nutrient Content

The result of two-way ANOVA (Table 6) showed that treatment and variety had a significant effect ($p< 0.01$) on tuber and shoot % N, Fe content, P content, and Zn content. Tuber Fe content was improved significantly ($P< 0.05$) by variety. A significant difference ($p<0.01$) was observed in the interaction between treatment and variety on tuber and shoot % N, Fe content, Zn content, and P.

Table 6*: Mean square of treatment, variety, and their interaction on shoot and tuber nutrient content*

Source of variation	N tuber	N shoot	Fe shoot	Fe tuber	Zn shoot	Zn tuber	P shoot	P tuber
Rep	0.0421^{ns}	0.2148^{ns}	632.27^{ns}	2152.8^{ns}	10704.2^{ns}	45.6714^{ns}	14.7801^{ns}	16.221n^{s}
Var	1.4222**	0.7581**	8140.8**	4334.2*	1466.2**	1600.29**	147.902**	790.987**
Trt	1.7677**	0.4228**	41009.7**	7320.83**	2794.2**	2796.58**	20.0946**	481.912**
Var.Trt	0.8859**	0.1712**	33606.9**	3864.07**	1556.4**	3178.14**	33.108**	318.943**
Error	0.0005	0.0151	3.721	56.16	277.1	0.3774	0.0918	0.1341
LSD (5%)	0.038	0.201	3.1704	12.3165	27.3607	1.0097	0.498	0.602
CV%	1.9	7	1.5	10.1	46.6	1.6	1.9	1.9

Note: *, **: statistically significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ probability level respectively; NS: Not Significant

Effect of PPB Inoculation on Potato Varieties' Macro and Micro Nutrient Content

The effect of treatment of PPB on potato macro and micro nutrient contents is shown on Table 7. The result demonstrated that tuber % N of the *Gudene* variety was significantly ($P < 0.01$) increased by inoculation of BSO5 (*Raoultella planticola/ornithinolytica*).

On the other hand, inoculation of the *Gudene* variety with STG5 *(Bacillus flexus)* does not show a statistically significant difference compared to the control. A significant difference ($P<0.01$) was observed in the interaction between HK1 (*Providencia rettgeri*) par with SBO4 (*Lysinibacillus sphaericus*) and AR2 (*Enterobacter aerogenes; Kleb. mobilis*) of *Bete* variety on shoot % N. However, a significant difference was not found in the inoculation of STG5 *(Bacillus flexus)* compared to the control.

The maximum shoot Fe (410.1 mg/kg) was observed on *Bete* inoculated with SBO4 (*Lysinibacillus sphaericus*), and the minimum shoot Fe (12.6mg/kg) was observed on uninoculated control (Table 7). A statistically significant difference was not found in the inoculation of this variety by STG5 compared with the control. *Bete* variety inoculated with SBO4 (*Lysinibacillus sphaericus*) and STG5 *(Bacillus flexus),* and *Bubu* variety inoculated with SLL1 (*Lysinibacillus fusiformis)* significantly ($P<0.01$) increased the tuber Fe compared with the control. On the contrary, a significant difference ($P<0.001$) was not found in the interaction between the *Bete* variety inoculated with HK1 (*Providencia rettgeri*) compared to the control.

A significant difference ($P<0.01$) was observed in the interaction between SLL1 (*Lysinibacillus fusiformis*) and *Bete* variety on Shoot Zn content. However, a significant difference was not found in the inoculation of HK1 (*Providencia rettgeri*) with *Gudene* and AR2 (*Enterobacter aerogenes; Kleb. Mobilis*) with *Bubu* variety compared with the control. The tuber Zn content was significantly ($P<0.01$) improved by the inoculation of AR2 (*Enterobacter aerogenes; Kleb. Mobilis*) on the *Bete* variety.

***Table 7**: Mean of treatment and variety on shoot and tuber nutrient uptake character*

Treatment	N tuber	N shoot	Fe shoot	Fe tuber	Zn shoot	Zn tuber	P shoot	P tuber
BT x Cont	0.294^{qr}	1.351^{l}	12.6^{t}	23.62^{f}	4.39^{e}	9.03^{q}	13.54^{i}	5.47^{m}
BTxHK1	1.572^{e}	2.358^{a}	117.0^{k}	23.62^{f}	32.44^{cd}	18.06^{n}	23.41^{a}	20.72^{f}
BTxAR2	0.881^{o}	2.299^{ab}	154.2^{i}	67.37^{d}	38.92^{bcd}	111.36^{a}	18.03^{e}	24.3^{d}
BTxBSO5	1.275^{h}	2.059^{cd}	22.1^{s}	119.86^{b}	40.94^{bcd}	57.19^{g}	22.51^{b}	17.13^{i}
BTxSBO4	1.225^{i}	2.304^{ab}	410.1^{a}	146.11^{a}	63.80^{b}	21.07^{m}	14.44^{h}	27^{c}
BTxBSO1	1.373 ^{g}	1.961^{cde}	97.8^{l}	67.37^{d}	31.23^{cde}	13.54^{p}	14.44^{h}	19.82^{g}
BTxSLL1	1.449^{f}	1.899^{def}	234.7^{e}	119.86^{b}	123.82^{a}	46.65i	18.03^{e}	14.44^{l}
BTxSTG5	1.123 ^{j}	1.367^{kl}	12.6^{t}	146.11^{a}	20.24^{de}	21.07^{m}	19.82^{d}	21.61^{e}
BU x Cont	0.327 ^{q}	1.367^{kl}	22.1^{s}	23.62^{f}	4.39^{e}	9.03^{q}	12.65^{j}	5.47^{m}
BU xHK1	1.078 ^{k}	1.765^{efgh}	126.2^{j}	67.37^{d}	34.71^{cd}	109.86^{b}	13.54^{i}	21.61^{e}
BU xAR2	0.927^{n}	1.562^{ijk}	88.0^{o}	49.87^{e}	4.39^{e}	30.10 ^{j}	18.03^{e}	17.13^{i}
BUxBSO5	1.029 ^{l}	1.863^{defg}	154.6^{h}	93.61^{c}	43.90^{bcd}	60.20 ^{f}	16.23^{f}	14.44^{l}
BUxSBO4	0.884^{o}	1.572^{hij}	297.1^{b}	93.61^{c}	47.77^{bc}	87.28^{c}	21.61^{c}	16.23^{j}
BUxBSO1	0.833^{p}	1.667^{ghi}	59.9^{q}	23.62^{f}	34.71^{cd}	30.10 ^{j}	23.41^{a}	5.47^{m}
BUxSLL1	1.765^{d}	2.108^{bc}	78.9^{p}	146.11^{a}	33.59^{cd}	54.18^{h}	18.03^{e}	17.13^{i}
BUxSTG5	1.032 ^{l}	1.375^{jkl}	88.5^{n}	49.87^{e}	32.44^{cd}	9.03^{q}	15.34^{g}	14.44^{l}
GU x Cont	0.277^{qr}	1.375^{jkl}	22.1^{s}	23.62 ^{f}	4.39^{e}	9.03^{q}	11.75^{k}	14.44^{l}
GUxHK1	1.589^{e}	1.390^{jkl}	214.0^{g}	67.37^{d}	4.39^{e}	46.65^{i}	13.54^{i}	21.61^{e}
GUxAR2	2.495^{b}	1.761^{efgi}	258.1^{d}	119.86^{b}	52.54^{bc}	24.08 ^{k}	13.54^{i}	20.72^{f}
GUxBSO5	2.685^{a}	1.757^{fghi}	229.3^{f}	67.37^{d}	43.90^{bcd}	13.54^{p}	12.65^{j}	65.56^{a}
GUxSBO4	1.275^{h}	1.569^{hij}	31.5^{r}	67.37^{d}	28.59^{cde}	61.70^{e}	14.44^{h}	32.38^{b}
GUxBSO1	2.108^{c}	1.667^{ghi}	268.1^{c}	67.37^{d}	38.92^{bcd}	22.57^{l}	13.54^{i}	15.34^{k}
GUxSLL1	0.987^{m}	1.875^{def}	88.9^{m}	49.87^{e}	37.89^{bcd}	15.05^{o}	14.44^{h}	18.03^{h}
GUxSTG5	0.294^{r}	1.618^{hi}	97.8^{l}	49.87^{e}	54.43^{bc}	70.73^{d}	13.54^{i}	15.34^{k}
LSD (5%)	0.038	0.201	0.0475	12.3165	27.3607	1.0097	0.498	0.602
CV%	1.9	7	4.1	10.1	46.6	1.6	1.9	1.9

BU: *Bubu;* BT: *Bete*; GU: *Gudene;* Different letters indicate significant differences at P≤0.05 according to the LSD test.

However, STG5 *(Bacillus flexus)* inoculated on the *Bubu* variety does not show a statistically significant difference over the control (Table 7).Interaction between treatment and variety was significant (P<0.01) on shoot phosphorous content of inoculated with BSO1 (*Serratia marcescens ss marcescens*) and HK1 (*Providencia rettgeri*) with *Bubu* and *Bete* varieties respectively. Similarly, the *Gudene* variety inoculated

with BSO5 (*Raoultella planticola/ornithinolytica*) significantly ($p<0.01$) increased the tuber phosphorous content by 77.97% compared to the control.

The percent of increase/decrease of inoculants demonstrated that inoculation significantly increased all the nutrient uptake parameters tested compared to uninoculated control. All isolates significantly increased tuber N, P, and Fe nutrient content of *Bete* by 76.17%, 72.59% and 65.44%, respectively. The shoot Zn and N content of *Bete* increased by 88.31 and 31.29%, respectively. The isolates also improved *Bubu* variety tuber N (76.17%), P (57.34%), and Zn (69.97%) as well as shoot phosphorous by 26.67%. On the other hand, the shoot Fe content of the *Gudene* variety improved by 77.97%.

In general, inoculation of potato varieties with SLL1 (*Lysinibacillus fusiformis*) and BSO5 (*Raoultella planticola/ornithinolytica*) improved the nutrient uptake of potato shoots and tuber by 60%. Other inoculants such as AR2 (*Enterobacter aerogenes; Kleb. Mobilis*), SBO4 (*Lysinibacillus sphaericus*), and BSO1 (*Serratia marcescens ss marcescens*) increased these parameters with an average higher than 40% compared to the uninoculated control plants. Thus, The *Bete* variety (62%) responded better to inoculation in terms of the significant number of uptake parameters followed by the *Bubu* (56%) and *Gudene* (51%) variety, respectively

Correlation Analysis of Growth Promotion, Yield Related and Nutrient Uptake Parameters of Plant Probiotic Bacteria Inoculant on potato.

Correlation Analysis of Yield-Related and Growth Promotion Parameters of Plant Probiotic Bacteria Inoculant on Potato

Correlation analysis of shoot dry weight, tuber dry weight, dry matter content (%), and root dry weight with other plant growth promotion characters revealed that shoot dry weight had a strong positive correlation (Fig.1) with leaf number (R=0.858), shoot fresh weight (R=0.753), plant height (r=0.656), stem thickness (R=0.552), internode length (R=0.418) and tuber fresh weight (R=0.542) whereas tuber dry weight showed a positive association with tuber fresh weight (r=0.860) and leaf area index (r=0.495). Root dry weight positively correlates with root fresh weight (r=0.803), shoot fresh weight (r=0.726), tuber number (r=0.540), shoot dry weight(R=0.530), and plant height (r=0.415). Moreover, dry matter content (%) had a strong positive correlation with starch content (r=0.99).

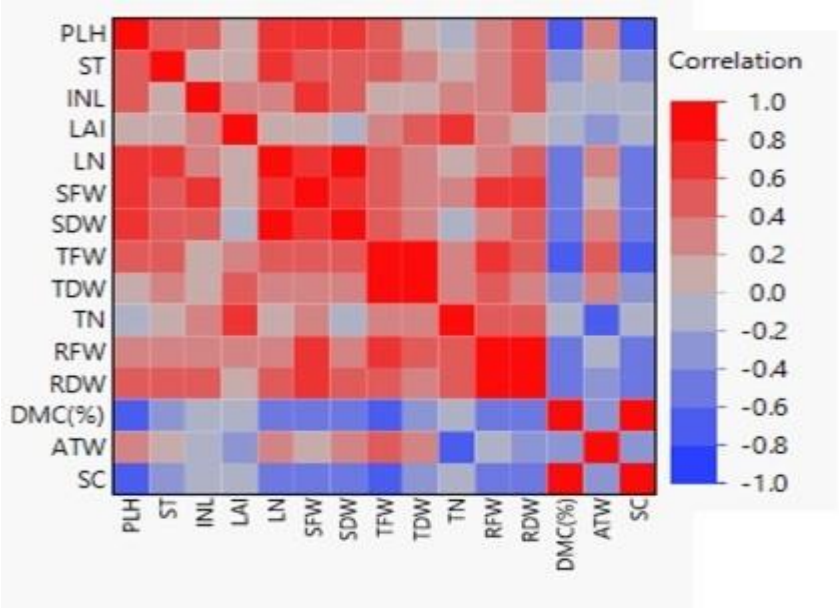


Fig 1. The correlation Map of Yield-Related and Growth Promotion Parameters

Correlation Analysis of Nutrient Uptake Parameters of Plant Probiotic Bacteria Inoculant on potato

Correlation analysis of tuber nitrogen, phosphorus, Iron, and Zink with shoot nitrogen, phosphorus, Iron, and Zink uptake (Fig.2) characters revealed that tuber nitrogen content had a strong positive correlation with

shoot iron (r=0.506), shoot nitrogen (r=0.329) and shoot zinc content (r=0.314); tuber phosphorous content showed a positive association with shoot iron (r = 0.31). Tuber iron content correlates with shoot nitrogen (r=0.348), shoot zinc (r =0.493), and shoot iron content (r=0.413). Furthermore, Tuber zinc content correlates with shoot nitrogen (r=0.267), shoot zinc (r =0.244), and shoot phosphorus content (r=0.294).

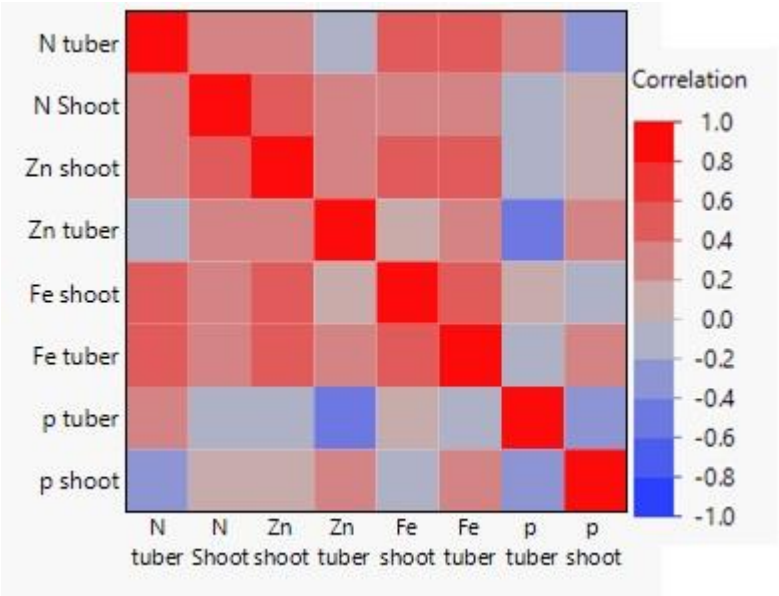


Fig 2. The correlation Map of Nutrient Uptake Parameters

Discussion

Natural soil contains a vast array of varied microbial populations that play a vital role in the recycling of organic materials and biogeochemical cycling processes, both of which facilitate the cultivation and growth of plants [31]. Plant probiotic bacteria isolates (PPB) significantly increased the surrogate agronomic characters except, LAI, PLH, INL, and RFW compared to uninoculated control plants. Inoculated potato varieties recorded increased number of tubers ranging from 22-67%. They also increased the number of tubers in the treated potato varieties ranging from 22-67% on the *Bete* variety; 17-33% on the *Bubu* variety and 8-25% on the *Gudene* variety compared to the control potato plants (Table 4). AR2 *(Enterobacter aerogenes; Kleb. mobilis)*, SLL1 *(Lysinibacillus fusiformis)*, and STG5 *(Bacillus flexus)* significantly improved the tuber's number of *Gudene* and *Bubu* varieties relative to the uninoculated control. Variation in the number of tubers reported following the inoculation of different varieties of potatoes with PGPR [32].Other studies have also confirmed that potato yield in terms of tuber numbers can be greatly enhanced by inoculants [33,34] .

The *Bete* variety inoculated with *SLL1 (Lysinibacillus fusiformis)* and SBO4 *(Lysinibacillus sphaericus)* improved tuber dry weights by 23.31 and 20.55% above the un-inoculated control, respectively. Corroborating to this finding, increased tuber dry weights was observed on potato plants inoculated with PGPR [32]. Other studies have also shown that potato growth, biomass, and yield can greatly be enhanced by Rhizobacteria treatments [33,34]. The root dry weights improved 50 and 49.99% above the un-inoculated control were shown by *Bete* variety inoculated with AR2 *(Enterobacter aerogenes (Kleb. mobilis))* and *SLL1 (Lysinibacillus fusiformis)*, respectively. Significant variations in root dry weights following inoculation of different varieties of potato plants with PGPR were reported [32]. In line with our findings, enhancement of potato growth, biomass, and yield were shown by Rhizobacteria treatments [33]. Inoculation of HK1 (*Providencia rettgeri*) and SLL1 (*Lysinibacillus fusiformis*) with *Bubu* and *Gudene* variety resulted in increased potato stem thickness relative to the uninoculated controls. This might be due to phytohormone production and nutrient competition [6,12].

The *Bete* variety inoculated with STG5 *(Bacillus flexus),* and the *Gudene* variety with SLL1 (*Lysinibacillus fusiformis*) enhanced the internode length and tuber fresh weight, respectively. Whereas the inoculation of HK1 (*Providencia rettgeri*) and SLL1 (*Lysinibacillus fusiformis*) with *Bete* and *Bubu* varieties increased the shoot's fresh weight. Likewise, HK1 (*Providencia rettgeri*) and SLL1 (*Lysinibacillus fusiformis*) with *Bete* and *Gudene* varieties significantly increase root fresh weight. This might be due to the N fixation and P solubilization capacity of the bacterial isolates utilized. Due to the impacts of N and P metabolic processes such as protein production, assimilate translocation, glucose metabolism, and enzyme conformation are reflected in strong vegetative growth [35].

Bete variety inoculated with AR2 *(Enterobacter aerogenes (Kleb.mobilis)),* BSO5 *(Raoultella planticola/ornithinolytica),* and BSO1 *(Serratia marcescens ss marcescens)* enhanced the average plant height compared to the uninoculated control. Pot trials by Oswad et al [34] , indicated that *in vitro* bacterial inoculation of potato plants also improved their growth. Recent observations were also made by Pathak et al [32] and Verma et al [26] following the inoculation of different varieties of potato plants with *Bacillus* and *Enterobacter* species as plant growth-promoting Rhizobacteria.

Inoculation of HK1 (*Providencia rettgeri*)Par with AR2 (*Enterobacter aerogenes*(*Kleb. mobilis*)) with *Bete* variety, and SLL1 (*Lysinibacillus fusiformis*) with *Bubu* and *Gudene* variety resulted in increased shoot dry weights relative to the uninoculated controls. Verma et al [26] reported potato tubers inoculated with *Enterobacter cloacae strain PGLO9* increased (69.93 %) the shoot dry weight over the control. Findings of bacterial inoculation effect of shoot dry weight on other crops like broccoli [36], and spinach [37] were also documented. Phytohormone produced by the inoculated plant probiotic bacteria may also influence root development by increasing the uptake of essential nutrients necessary for plant growth [38]. *Bete* variety inoculated AR2 (*Enterobacter aerogenes* (*Kleb. mobilis*)) and SBO4 *(Lysinibacillus sphaericus)* resulted in increased potato leaves and leaf Area Index relative to the uninoculated control. Oswad et al [34] reported similar observations have been made for potato leaves. This might be due to the increased Nitrogen uptake through nitrogen fixation by inoculated PPB isolates. This increase has a positive effect on the chlorophyll concentration, the photosynthetic rates, the leaf expansion, the total number of leaves, and the dry matter accumulation [37]. In the potato, adequate chlorophyll concentration is necessary for photosynthesis and formation of CHO which are the major components of tubers [39]. Studies showed that beneficial microbes and inoculants can enhance plant growth indirectly by enhancing Chl quantities [38].

The quantification of N in the shoot and tubers of the potato variety revealed that the highest concentrations of tuber N were recorded for the *Gudene* variety inoculated with BSO5 (*Raoultellaplanticola/ornithinolytica*). Similarly, HK1 (*Providencia rettgeri*), AR2 (*Enterobacter aerogenes* (*Kleb. Mobilis*)) and SBO4 (*Lysinibacillus sphaericus)* increased shoot N concentration. According to Jha et al [40], the total plant N quantities increase due to BNF and increased availability of N in the rhizosphere. Variety *Gudene* with BSO5 *(Raoultella planticola/ornithinolytica*) showed significantly higher tuber P contents than the un-inoculated control by approximately 77.97%, which is a clear indication of enhanced P uptake in the treated plants. Phosphorus solubilizing inoculants enhanced P availability in the Grapevine rhizosphere soils and also increased its uptake in the treated pots [41]. Increased P uptake by crops under inoculation has been reported in several studies in growth chambers and greenhouses [42–44]. The inoculation of seed potato varieties with BSO1 *(Serratia marcescens ss marcescens)* and HK1 (*Providencia rettgeri*) resulted in shoots with a higher P content (23.41 mg kg^{-1}) corresponding to an increase of 42.16-45.96% above the un-inoculated control. Increased p uptake was obtained in peanuts following inoculation with *Serratia* and *Enterobacter* species [45].

Inoculation of AR2 *(Enterobacter aerogenes (Kleb. Mobilis))* with *Bete* variety enhanced the tuber Zn contents significantly by 91.89% relative to the un-inoculated control. This increase may be attributed to the ability of inoculants to mobilize Zn in the potato rhizosphere (46). Rhizobacteria-mediated Zn increments in plant tissues were reported in *Capsicum annuum* L [9], wheat, and sugarcane [47]. However, studies on potato Rhizobacteria Zn solubilization are scarce [6].

Significantly higher potato shoot Zn content was recorded from the treatments with SLL1 *(Lysinibacillus fusiformis)* corresponding to an increment of 96.45% above the uninoculated control. Hence this was the best inoculant in terms of enhancing potato Zn uptake which enhances plant Zn uptake and elevates the Zn concentrations in plant tissues. Microbial transformations of unavailable Zn forms in soil to plant-available ones by ZSB can influence the mobilization and uptake of Zn in edible portions [47]. The present study

showed that inoculation of SBO4 *(Lysinibacillus sphaericus)* and STG5 *(Bacillus flexus)* with the *Bete* Variety and SLL1 *(Lysinibacillus fusiformis)* with the *Bubu* variety increased the potato tuber Fe contents by approximately 83.54% above the uninoculated control. However, significantly higher shoot Fe content (410.1 mg kg^{-1}) was recorded by SBO4 *(Lysinibacillus sphaericus)* corresponding to 94.49% increments above the un-inoculated control. Reports support this finding are available on Rhizobacteria-mediated Fe increments[48].

Conclusion

Plant probiotic Bacterial inoculation significantly increased the surrogate agronomic character by 20-40% compared to the uninoculated control, except LAI on all varieties and PLH, INL, and RFW on one of the varieties. All varieties combined, the trend showed that isolates HK1 (*Providencia rettgeri*) and SLL1 *(Lysinibacillus fusiformis)* were the best performers in improving the surrogate agronomic characters of potato varieties followed by isolates AR2 *(Enterobacter aerogenes (Kleb.mobilis))* and STG5 *(Bacillus flexus)*. All isolates significantly increased the SDW of *Bete* variety ranging from 29-37% (mean 33%) compared to control; whereas fewer isolates; AR2 *(Enterobacter aerogenes(Kleb.mobilis))*, SBO4 *(Lysinibacillus sphaericus)*, and SLL1 *(Lysinibacillus fusiformis)* increased SDW by 14, 14 and 33%, respectively. It is interesting to note that SLL1 increased the SDW of all varieties. The *Bete* variety performed better by producing more tubers (TN) with a mean of 32% over control plants followed by the *Bubu* and *Gudene* varieties, with a mean TN of 22%, and 17% respectively. All isolates significantly increased tuber N, P, and Fe nutrient content of *Bete* by 76.17%, 72.59% and 65.44%, respectively compared to the control plants. In general, inoculation of potato varieties with SLL1 *(Lysinibacillus fusiformis)* and BSO5 *(Raoultella planticola/ornithinolytica*) improved the nutrient uptake of potato shoots and tuber by 60% followed by a 40% increment by AR2 *(Enterobacter aerogenes (Kleb. Mobilis)*, SBO4 *(Lysinibacillus sphaericus)*, and BSO1 *(Serratia marcescens ss marcescens)*. The *Bete* variety responded better to inoculation (62%) in terms of the significant number of nutrient uptake parameters. Further research and experimentation can advance understanding of the specific mechanisms through which plant probiotic bacteria exert their beneficial effects for potato cultivation and sustainable Agricultural practice.

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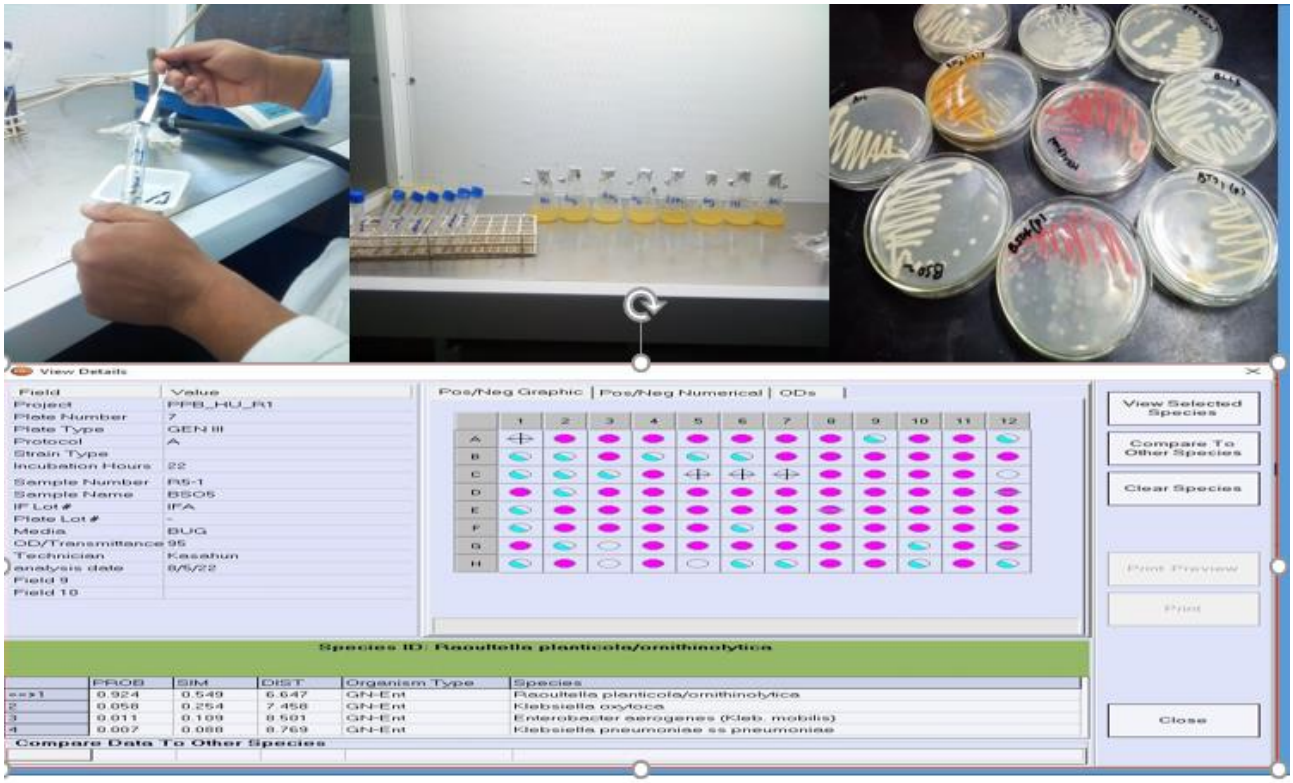
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Supplementary Material



Supplementary Figure 1: Isolation and Identification



Supplementary Figure 2: Greenhouse Experiment for potato growth promotion