

BENEFICIAL EFFECTS OF BACTERIAL ENDOPHYTES ISOLATED FROM POTATO ROOTS AND TUBERS ON NUTRIENT SOLUBILIZATION

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ABSTRACT: The application of agrochemicals to improve nutrient uptake in plants has several negative effects on soil health. The use of plant growth-promoting rhizobacteria (PGPR) with nutrient solubilizing properties has become an attractive alternative to the synthetic chemicals. Endophytic bacteria have the potential to be utilized effectively in integrated nutrient management for crops. Being a vegetatively propagated crop, potato is continuously perpetuating a large number of microorganisms in its tuber as well as in its roots. Bacteria from potato tubers and roots were isolated and tested for their ability to solubilize Zn, P, and K and to produce siderophores on zinc, pikovskaya, aleksandrow, and chromo azurol S medium, respectively by serial dilution plate technique. A pot experiment was also conducted to study the effect of isolated endophytes on potato plant growth and yield. Among all, isolate PSB2 was found the best withstanding high pH and NaCl salt concentration. At seven days after inoculation (DAI) on respective media, it produced siderophore, IAA (9.79 g/ml), and solubilized phosphate (21.20 P g/ml). All six bacterial isolates showed a positive impact on growth and growth attributing characteristics in potato *cv. Kufri Jyoti*. Treatment T11 received recommended dose of nitrogen, phosphorus, and potassium (RD+NPK) + Zn in soil and a combination of endophytes isolates seed tuber treatment recorded maximum germination percent, plant height, fresh and dry biomass, and tuber weight which was significantly superior over un-inoculated control and at par with 100 % recommended dose of fertilizers (T1) suggesting possible saving in 25% of chemical fertilizers *viz.* phosphatic, potassium and zinc requirement in potato cultivation.

KEYWORDS: Potato, endophytes, zinc solubilizers, potassium solubilizers, siderophore

INTRODUCTION

Endophytes live inside the plant in a non-pathogenic way and develop a mutualistic relationship (Montanez *et al.*, 2012). Endophytes produce secondary metabolites that protect plants against pathogens and pests. Some endophytes produce growth hormones like indole acetic acid (IAA) (Kumar *et al.*, 2021a), ethylene, and cytokinin. These microbes can be used in sustainable agriculture practices as this improves crop yield without harming the environment (Singh *et al.*, 2022). If endophytes are to be used in crop production systems, it will be necessary to manipulate bacterial populations in soils and plants. However, their role in

nutrient accumulation is not much explored. Hence, the study of endophytes and their compounds in crop production is needed in the present scenario of environmental pollution and climate change. Different parts of the potato are host to an abundance of endophytes. Both tubers and soil contain beneficial bacteria necessary for rhizosphere assembly and microbiota-mediated nutrient management. This can be converted into potato and ecosystem health microbiome management techniques.

Endophytes enhance the uptake of nitrogen for their host plant. In association with the host plant, this microorganism fixes atmospheric nitrogen in the presence

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of the nitrogenase enzyme (Montanez *et al.*, 2012). Soil application of phosphatic fertilizers makes available only a small amount of soluble phosphorus fraction for plant uptake. Endophytic microorganisms produce different acids and compounds such as phosphatases and phytases that hydrolyze organic phosphorus compounds that help the solubilization of fixed phosphorus (Kumar *et al.*, 2021b). Endophytic populations have been extracted from cactus, strawberries, sunflower, soybeans, and other legumes found to be mineral phosphate solubilizers (Dias *et al.*, 2009).

Potassium (K) deficits, on the other hand, are an issue because K depletes rapidly from soils due to crop absorption, runoff, leaching, and soil erosion (Sheng and Huang, 2002). Rock phosphate (P) and K materials are less expensive sources of P and K. Nevertheless, most of them are not readily available to plants since the minerals are released slowly, and their use as fertilizer frequently results in negligible yield improvements of existing crops (Zapata and Roy, 2004). Phosphorus solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB), and zinc solubilizing bacteria (ZSB) have been utilized to enhance the solubilization of fixed P, K, and zinc (Zn) because they convert insoluble forms of nutrients into those that are soluble and available for plant growth (Nahas *et al.*, 1990). This conversion occurs via acidification, chelation, and exchange processes (Gerke, 1992) and generates powerful organic acids in the periplasm (Alexander, 1977), which are markers for routine isolation and selection of solubilizer (Illmer *et al.*, 1995). In sustainable agriculture, bio fertilizers have been employed as a source to boost plant nutritional content. Experiments were conducted to determine the efficacy of phosphate solubilizing bacteria *Bacillus megaterium* var. *phosphaticum* and

potassium solubilizing bacteria *Bacillus mucilaginosus* implanted in nutrient-deficient soil containing pepper and cucumber.

Utilizing fertilizers in conjunction with bio fertilizers enhanced the availability of P, K, and Zn in the soil and the uptake of nitrogen (N), P, K, and Zn by the shoot and root. *Bacillus mucilaginosus*, for example, has been shown to improve K availability in soils and mineral content in plants (Sheng *et al.*, 2002). An integrated application of rock P and K materials with co-inoculation of bacteria that solubilize them could provide a more rapid and consistent supply of P and K for optimal plant growth. These endophytes can be used as efficient biological plant growth promoters in sustainable crop production. Being a vegetatively propagated crop, the potato continuously perpetuates a large number of microorganisms in its tuber as well as in its roots. Hence, there is great scope for the identification of novel endophytes from potato tubers along with roots. To investigate their impact on potato plant growth parameters, we studied bacterial endophytes from the tubers and roots grown under field conditions.

MATERIALS AND METHODS

Potato tubers and roots were collected from long term fertilizer experiment plot at ICAR-Central Potato Research Institute, Regional station, Meerut, Uttar Pradesh, India during Rabi season (year 2018). Freshly harvested potato roots were rinsed with tap water to remove any remaining soil. The surface of the roots and tubers were sanitized with 70% ethanol for 30 seconds and 2% sodium hypochlorite for five minutes, and then they were washed twice with sterilized distilled water (Elbeltagy *et al.*, 2000). Roots and tubers were chopped aseptically into 1–2 cm sections, macerated with 0.8% saline solution and quartz sand, and then

decimally diluted in 0.8% saline solution. Endophytic isolates with the following plant growth enhancing properties have been isolated: siderophore producer, which plays a significant role in plant iron nutrition and phytopathogen repulsion. Microorganisms that solubilize zinc, phosphate, and potassium mobilize soil nutrients and make them readily available to crop plants. The isolates were purified by subculture on their respective media, and a stock culture was kept at 4–5 °C for future research.

Characterization of isolated bacteria

Standard protocols were used to determine the morphological properties, such as shapes, Gram reaction (Hucker and Conn, 1923), opacity, margin characteristics, and motility (Whittenbury, 1964) of all isolates.

Screening of bacterial isolates for their P, K, and Zn solubilization

On agar plates

Bacterial strains were evaluated for their ability to solubilize potassium, phosphorus, and zinc on modified Aleksandrov medium plates supplemented with mica powder (2 g/L), Pikovskaya Agar medium plates supplemented with calcium phosphate, and zinc solubilizing agar medium plates supplemented with zinc oxide, respectively. On the basis of the solubilization zone generated on medium plates after seven days of incubation at 28°C, the solubilization patterns of several strains were evaluated. The results were expressed as follows, according to Nguyen et al. (1992): $SE = \text{Solubilization Diameter} / \text{Growth Diameter} \times 100$.

Quantitative assessment of P, K, and Zn solubility

In liquid culture

100 ml Erlenmeyer flasks containing 20 ml of Pikovskaya (PKVK) Agar medium

were inoculated with an inoculum of bacterial isolates and incubated for seven days at 28 °C. The cultures were harvested by centrifuging at 5000 rpm for 20 minutes, and the phosphorous content in the culture supernatant was evaluated using the para molybdenum blue method (Olsen and Sommers, 1982). At 600 nm, a spectrophotometer was used to determine the color produced during P estimation (Naik *et al.*, 2008). Further, a quantitative examination of zinc solubilized in a liquid medium containing 5 mM zinc oxide was conducted. By inoculating 1 percent cultures of each endophyte in liquid medium and incubating at 30 °C, 150 rpm for seven days, the amount of zinc solubilized was determined by Atomic absorption spectrophotometer. As a control, uninoculated medium for each zinc salt was used. Mica was supplied as the sole potassium source to the liquid Aleksandrov medium to test the isolate's capacity to solubilize potassium. A quantitative determination of potassium solubilization was carried out in Erlenmeyer flasks containing 100 mL of Aleksandrov medium and inoculated in triplicate with KSB1 and KSB2. As a control, autoclaved, uninoculated media were used. The flasks were incubated in a BOD incubator at 28°C. With a flame photometer, the dissolved potassium in the culture's supernatant, which was made by filtering the culture with a Whatman filter number 42, was measured. All the tests were conducted in five replications.

Identification of bacteria using 16S rRNA sequences

Morphological study of microorganisms is usually the initial step of identifying endophytes. However, molecular techniques are needed to confirm their existence. The following steps were followed for molecular identification of endophytes:

Isolation of DNA:

Total genomic DNA was extracted as described by Chen and Kuo (1993). The cultures were grown at 28 ± 2 °C for 48 h with vigorous shaking. Each culture (1.5 ml) was harvested by centrifugation for 3 min at 12,000 rpm. The cell pellet was re-suspended and lysed in 300 µl of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS, 20 µg RNase A) by vigorous pipetting and incubated for 30 min at 37 °C. To remove most proteins and cell debris, 100 µl of 5 M NaCl solution was added and mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10 min at 4 °C. After transferring the clear supernatant into a new vial, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 12,000 rpm for 3 min, the extracted supernatant was transferred to another vial and the DNA was precipitated with 100 % ethanol, washed twice with 70 % ethanol, dried in speed-vacuum, and re-dissolved in 50 µl of TE buffer.

PCR amplification of the 16S rRNA gene

Identification of the bacterial species was based on the sequencing of the 16S rRNA using the primers 27 F and 1492 R (Lane, 1991). Genomic DNA was extracted by the phenol/chloroform extraction method. The 16s rRNA gene was amplified using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG -3') and 1492R (5'-GGTACC TTG TTA CGA CTT-3') (Table 4). The reaction was carried out in a 50 microliter reaction mixture containing 1.5 mM MgCl₂, 2.5 mM of each dNTP, 25 pmoles of forward and reverse primers, 50 mg of DNA template, and 3U Taq DNA polymerase with its reaction buffer.

DNA extraction from gel (Gel elution)

The amplicons were gel eluted using the Min-elute gel elution kit following the manufacturer's instructions (Qiagen). Agarose 1.2 g was used to make the gel in 100 ml of 1 × TAE buffer. The solution of agarose and 1 × TAE buffer was heated up to 1 min to dissolve agarose. When cooled a bit, the gel was poured into the gel cast, the comb was fixed, and the gel was allowed to solidify for 20 min. Each sample prepared for running on gel had a volume of 40 µl mixed with 3 µl of loading dye. A 100 bp ladder was used to ascertain the size of the band. The gel was allowed to run for 40 min at 90 V. The bands on the gel were visualized and cut under a UV transilluminator and quantified at 260 nm using a spectrophotometer. The purified 16S rDNA amplicons were sequenced on an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, CA, USA) (Sagar *et al.*, 2014).

Analysis of 16S rRNA sequences

The sequencing of 16S rRNA was performed using both forward and reverse primers. The generated sequences were aligned using the BioEdit software, V. 7.2.5, and compared with NCBI databases and sequences showing [99 % similarity by the Nucleotide Basic Local Alignment Search Tool (BLAST) programme available at the National Center for Biotechnology Information (NCBI) BLAST server (www.ncbi.nlm.nih.gov/BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST>) (<http://www.ncbi.nlm.nih.gov/BLAST>) (<http://www.ncbi.nlm.nih.gov/BLAST>) The nucleotide sequences determined in this study have been deposited in the Gene Bank database and accession numbers have been obtained.

Effects of endophytes isolates on potato plants

Pot experiments were carried out at ICAR-Central Potato Research Institute,

Shimla, Himachal Pradesh, India during the year 2019 in a glass house in a completely randomized design (CRD) with three replicates. To determine the efficacy of the identified bacterial isolates as bio inoculants, sterilized soil and sand were combined in a 1:1 ratio and placed in plastic pots with a width of 12 cm. The potato tubers of cv. *Kufri Jyoti* were cut into pieces and left to dry for two days. Then, ten percent arabic gum was used to coat the pieces with selected isolates that had been grown in liquid culture medium for fifteen minutes. The pieces were then left to dry for fifteen minutes. Potato tubers were planted in pots below the soil's surface, faintly irrigated, and the remaining other activities were followed as per standard recommended agronomic package and practices of the region. After 30 and 60 days, potato plants were collected and measured for various growth parameters, including germination percentage, plant height (cm) at 30, 45, and 60 days after sowing (DAS), shoot length, fresh and dry weight for shoot, and tuber weight (g) at 90 DAS. Phosphorus, potassium, and zinc contents in shoots and tubers were determined according to the methods described by AOAC (2005). Treatment details of the experiment are presented in Table 1.

2.6 Statistical analysis

Analysis of variance (ANOVA) and LSD test were carried out for the acquired results according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Characterization of bacterial isolates

The colonies of the PSB1 isolates were regular, slightly raised, smooth and slimy, and opaque. The cells of all the isolates were gram-positive and short to long rods (Table 2). The colonies of the PSB2 isolates were irregular, convex, smooth and slimy, and opaque. The

Table 1 - Treatment details to evaluate endophytic isolates efficiency in nutrient solubilization, plant growth and yield under in vivo conditions.

Treatment No	Nitrogen, phosphorous, potassium, Zinc Chemical fertilizer (N, P ₂ O ₅ , K and Zn kg/ha)	B	Biofertilizer (B)*
T1	100%RDF		
T2	50%RDF		
T3	75%RDF	B1	PSB1
T4	75%RDF	B2	KSB1
T5	75%RDF	B3	ZSB1
T6	75%RDF	B4	PSB 2
T7	75%RDF	B5	KSB2
T8	75%RDF	B6	ZSB2
T9	75%RDF	B7	PSB1+KSB1+ ZSB 1
T10	75%RDF	B8	PSB2+KSB2+ ZSB 2
T11	75%RDF	B9	PSB1+KSB1+ ZSB 1+ PSB2+ KSB2+ ZSB 2

colonies of the KSB1 isolates were regular, slightly raised, smooth, and opaque. The colonies of the KSB2 isolates were irregular, convex, smooth, and opaque. The colonies of the ZSB1 isolates were regular, convex, smooth, and transparent. The colonies of the ZSB2 isolates were regular, undulated, convex, smooth, and opaque.

Sequencing of the 16S rRNA gene and identification of native potential isolates

The partial 16S rRNA gene sequence of 1500 bp was determined, and the output data were saved in FASTA format as detailed in Supplementary Figure: S1. The generated sequences were analysed with BLAST (Basic Local Alignment Search Tool) to identify the cultures and to locate the closest match of the cultures; the result is provided in the Table 3.

Isolation of endophytic bacteria and solubilization index

The present study was conducted to isolate and characterize P, K, and Zn

Table 2 Morphological characteristics of the endophytic bacterial isolates from potato roots.

Bacterial isolate	Shape	Gram reaction	Margin characteristic	Elevation	Opacity
PSB1	Regular, short rods	+ve	Entire	Slightly raised	Opaque
KSB1	Regular, short rods	+ve	Entire	Slightly raised	Opaque
ZSB1	Regular, short rods	+ve	Entire	Convex	Transparent
PSB2	Irregular, short rods	+ve	Undulated	Convex	Opaque
KSB2	Irregular, big rods	+ve	Entire	Convex	Opaque
ZSB2	Regular, short rods	+ve	Undulated	Convex	Opaque

Table 3 Identification of phosphorus, potassium, zinc solubilizing isolates by 16S rRNA sequencing.

Isolate Code	Species	Accession number	Similarity
PSB1	<i>Bacillus safensis</i>	MT576065	100%
KSB1	<i>Pseudomonas</i> sp.	MT576051	96%
ZSB1	<i>Bacillus cereus</i>	MT576024	100%
PSB2	<i>Bacillus velezensis</i>	MT575768	99%
KSB2	<i>Lelliottia amnigena</i>	MT573876	98%
ZSB2	<i>Pseudomonas granadensis</i>	MT573221	99%

solubilizing strains of endophytic bacteria isolated from potato tubers and roots. A total of 25 rhizobacterial isolates were isolated from tubers and roots of potatoes. These isolates were purified and stored at a low temperature for further experimentation. Six out of these twenty-five rhizobacterial isolates showed strong solubilization of insoluble P, K, Zn compounds during the agar plate assay and were coded as PSB1, KSB1, ZSB1, PSB2, KSB2 and ZSB2. Results obtained from *in vitro* screening of selected isolates for their ability to solubilize potassium monohydrogen phosphate, mica, and zinc oxide (ZnO) revealed that these isolates solubilization index for P, K, and zinc ranged from 1.25-3.12, 1.38-2.70, and 1.22-3.00, respectively. P solubilizing isolates PSB1, PSB2, K solubilizing isolates KSB1, KSB2, Zinc solubilizing isolates ZSB1 and ZSB2 showed maximum P, K, Zn solubilization zone and were statistically at par with each other (Table 4, Figure 1). Similar result was found by Mumtaz *et al.* (2017) where zinc

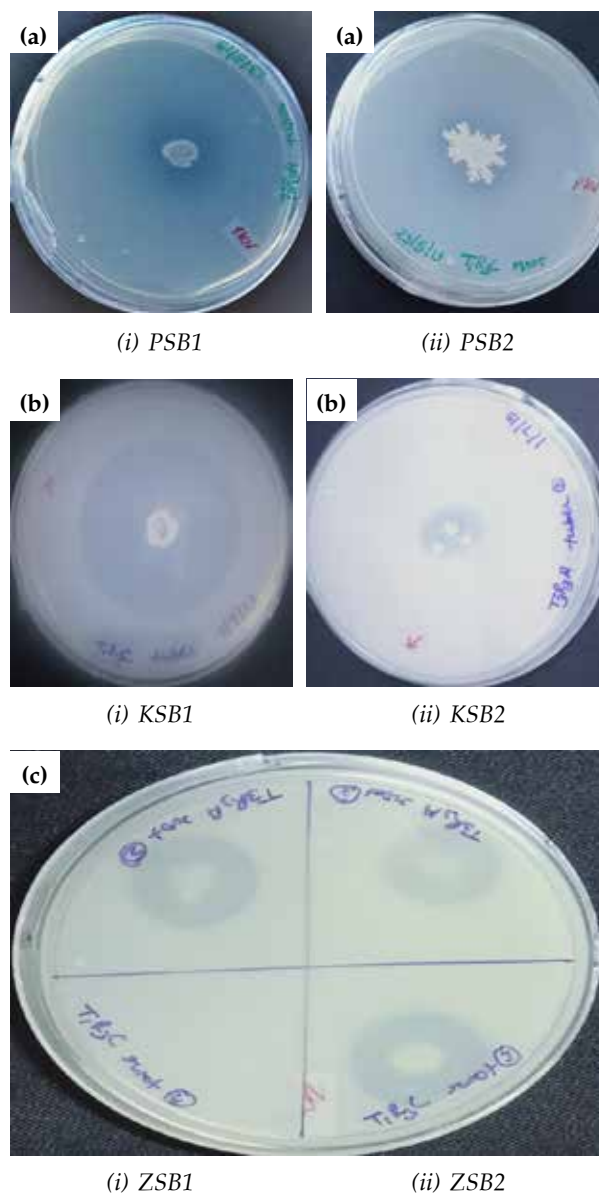


Figure 1(a) Phosphorus solubilizing bacteria, (b) Potassium solubilizing bacteria, (c) Zinc solubilizing bacteria

Table 4 Phosphorus, potassium and zinc solubilization index (PSI, KSI, ZnSI) of endophytic isolates after 7 days of inoculation.

S. No.	Isolates No.	PSI	KSI	ZnSI
1	PSB1	3.12 ^{a*}	1.38 ^e	1.22 ^f
2	KSB1	1.82 ^c	2.70 ^a	1.95 ^c
3	ZSB1	1.39 ^e	1.62 ^d	2.57 ^b
4	PSB2	2.90 ^b	2.08 ^b	1.67 ^e
5	KSB2	1.67 ^d	2.62 ^a	1.81 ^d
2q6	ZSB2	1.25 ^e	1.88 ^c	3.00 ^a

*Means with the same letter are not significantly different at ($p = 0.05$).

solubilizing bacteria produce 2.89-3.88 and phosphorus producing bacteria produce 2.33-4.03 solubilization index.

Zinc, Phosphorus, Potassium Solubilization Assay

Estimation of P in the PKVK broth revealed that all the strains released P from tri calcium phosphate (TCP). At 7 days after inoculation (DAI), isolates PSB1 and PSB2 had the highest available phosphate levels of 82.86 mg/L and 76.08 mg/L, respectively, followed by KSB1 and KSB2 (Table 5). Estimation of K in the mica broth revealed that all the strains released K from mica. Isolates KSB1 and KSB2 recorded maximum available potassium of 64.19 mg/l and 55.27 mg/l, respectively, at 7 DAI, followed by ZSB1 and ZSB2. The

Table 5 Test of phosphorus, potassium, and zinc solubilization ability of endophytic bacteria in broth was recorded after one weeks of incubation.

S. No.	Isolates No.	P mg/l 7 DAI	K mg/l 7 DAI	Zn mg/l 7 DAI
1	PSB1	82.86 ^{a*}	27.60 ^e	17.76 ^e
2	KSB1	52.28 ^c	64.19 ^a	20.75 ^d
3	ZSB1	13.44 ^e	49.18 ^c	28.24 ^b
4	PSB2	76.08 ^b	30.36 ^e	22.41 ^c
5	KSB2	29.70 ^d	55.27 ^b	18.26 ^e
6	ZSB2	11.29 ^e	40.28 ^d	33.62 ^a

*Means with the same letter are not significantly different at ($p = 0.05$).

minimum potassium was utilized by PSB1 and PSB2. Estimation of Zn in the zinc oxide broth revealed that all the strains released Zn from ZnO. Isolates ZSB1 and ZSB2 recorded maximum available zinc of 28.24 mg/L and 33.62 mg/L, respectively, at 7 DAI, followed by PSB2 and KSB1 (Table 5). The present findings establish that phosphate, potassium, and zinc solubilization can improve the availability of phosphorus, potassium, and zinc in the crop rhizosphere. It has been found that a wide variety of soil bacteria and fungi can solubilize different types of precipitated P (Kucey *et al.*, 1983; Singh *et al.*, 2017; Lal *et al.*, 2022). These organisms are dominated by *Bacillus*, *Pseudomonas*, *Penicillium*, and *Aspergillus* spp. According to a study, the potassium-solubilizing bacteria *B. mucilaginosus* can dissolve rock K mineral powders like mica, illite, and orthoclases by making and releasing organic acids (Ullaman, 1996).

pH Tolerance

PSB2 isolates have a wider pH (3-9) tolerance than other isolates, including PSB1, KSB1, ZSB1, PSB2, KSB2, and ZSB2. pH range (5-9) was observed for isolates KSB1 and KSB2 (Table 6). Bacterial growth was seen at pH 3 to 9 by isolates, but luxurious growth was observed between pH 5.0 and 7.0 for all the isolates. Gopalakrishnan et al. (2012) reported SRI-156 (*P. plecoglossicida*; NCBI Accession Number: JQ247008) as having the ability to tolerate pH between 5.0 and 13.0. In general, bacterial growth is higher in the pH range of 5.5-8.5.

Salt Tolerance

All the tested isolates showed growth at salt concentrations of 1-2 percent. Growth of isolates PSB2 observed at higher salt concentration showing capacity to tolerate up to 5 per cent compared to other isolates

Table 6 pH tolerance of endophytic isolates.

S. No.	Isolates No.	pH							
		2	3	4	5	6	7	8	9
1	PSB1	-	-	+	+	+	+	+	-
2	KSB1	-	-	-	+	+	+	+	+
3	ZSB1	-	-	+	+	+	+	+	-
4	PSB2	-	+	+	+	+	+	+	+
5	KSB2	-	-	-	+	+	+	+	+
6	ZSB2	-	-	+	+	+	+	+	-

(Table 7). For all isolates, 1 percent salt concentration resulted in the best growth. Gopalakrishnan *et al.* (2012) reported that *P. plecoglossicida* has the ability to tolerate salinity up to 6 per cent. Kuarabachew *et al.* (2007) observed that *P. fluoresces* can tolerate NaCl concentrations up to 3 percent in nutrient medium.

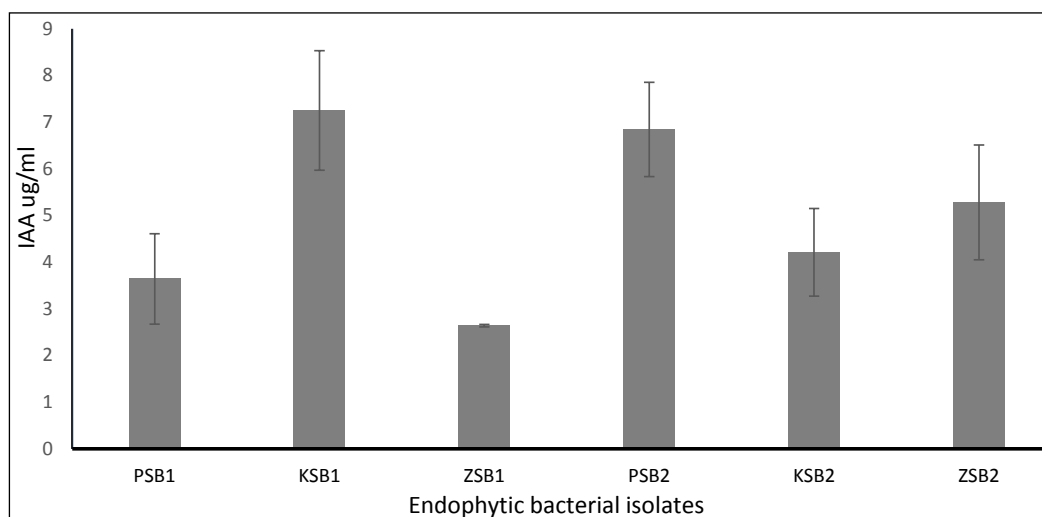
Estimation of growth hormone IAA

All six examined isolates cultured in a culture medium contained tryptophan as a carbon source for IAA formation, as measured by the Salkowski reagent and spectrophotometer in the range of 2.64 to 7.25 ug/ml IAA (Figure 2). The higher concentration of IAA was obtained by the

Table 7 Salinity tolerance of endophytic isolates.

S. No.	Isolates No.	Salinity %				
		1	2	3	4	5
1	PSB1	+	+	+	-	-
2	KSB1	+	+	-	-	-
3	ZSB1	+	+	+	+	-
4	PSB2	+	+	+	+	+
5	KSB2	+	+	+	-	-
6	ZSB2	+	+	-	-	-

KSB1 strain at 7.25 ug/ml, followed by the PSB2 strain at 6.84 ug/ml. Estimation of IAA in the medium revealed that all the strains released IAA in tryptophan nutrient broth. Sakthivel and Karthikeyan (2012) carried out quantitative estimation of IAA

**Figure 2** Estimation of IAA from endophytic isolates.

production by 30 bacterial isolates obtained from *Coleus forskohlii* rhizospheric soil of Perambalur and Salem districts in Tamil Nadu. IAA production by *Pseudomonas spp.* isolates ranges from 5.00–7.60 µg/ml, followed by *Azospirillum spp.* (3.10–6.80 µg/ml), *Azotobacter spp.* (3.00–6.30 µg/ml) and *Bacillus spp.* (2.40–5.80 µg/ml).

Detection of Siderophore

Out of six endophytic bacteria, only four were able to produce a yellow color zone, which indicates the siderophore production capacity of isolates, wherein isolates PSB1, PSB2, KSB2 and ZSB1 positive siderophore (Table 8). On Chrom Azurol S agar, Dave and Dube (2000) also isolated six rhizobacterial *P. fluorescens*. Two of the six isolates produced the most siderophore. Sakthivel and Karthikeyan (2012) detected siderophore production and among the 30 isolates belonging to *Pseudomonas spp.*, *Azospirillum spp.*, *Bacillus spp.*, and *Azotobacter spp.*, produced siderophore. Siderophore chelates iron and other metals and contributes to disease suppression by conferring competitive advantages to biocontrol agents.

Tuber inoculation efficacy of endophytic isolates on potato cv. Kufri Jyoti

A pot trial was carried out with a 100% recommended dose (full RD NP) of chemical fertilizer, i.e., (180 kg N/ha + 100 kg P₂O₅/

ha + 80 kg K₂O/ha + 25 kg/ha ZnSO₄ and ¾ RD (135 kg N/ha + 75 kg P₂O₅/ha + 45 kg K₂O/ha + 18.75 kg ZnSO₄ /ha) as urea and SSP to study the effect of various endophytic bacterial isolates on Potato cv. Kufri Jyoti and also to demonstrate phosphorus, potassium, and zinc savings in potato by bio-inoculants (Table 1).

Effect of endophytic isolates on potato plants germination and height

Data revealed that tuber inoculation of all the isolates significantly influenced germination per cent at 20 days after sowing (DAS). Among the different treatments of endophytic bacterial isolates, T11 and T9 recorded maximum germination per cent at 20 DAS, which was significantly superior to T1 (Figure 3). Endophytic bacteria induce the growth of plants as compared with uninoculated treatments. The results revealed that tuber inoculation of all the isolates significantly influenced plant height at 30, 45, and 90 DAS. Among the different strains of endophytic bacteria, combined application of isolates recorded maximum plant height at 30, 45, and 60 DAS, respectively, which was significantly superior over the un-inoculated control (Table 9).

Application of 75% fertilizer with endophytes significantly influenced plant height, i.e. T11 recorded 19.31, 46.25, and 67.22 cm, average plant height at 30, 45, and 90 DAS, respectively, which was at par with T10 (18.27, 39.36, and 58.05cm, respectively). This suggests combining the application of 75% of chemical fertilizers with bio fertilizers in potato cultivation enhances crop growth. A study conducted by Deivanai et al. (2014) on rice seedlings found that the inoculation of mangrove endophytic bacterial isolates has delayed the germination in both the pretreatments of rice seeds and increased the shoot length. A similar result was found

Table 8 Estimation of siderophore production by endophytic bacterial isolates.

S. No.	Isolates No.	Siderophore Detection
1	PSB1	+
2	KSB1	-
3	ZSB1	+
4	PSB2	+
5	KSB2	+
6	ZSB2	-

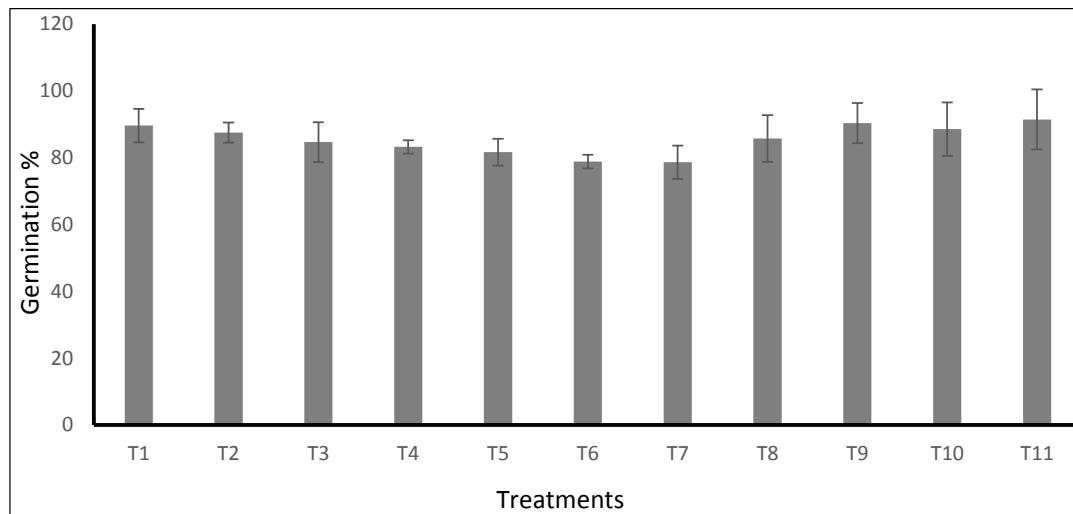


Figure 3 Inoculation efficiency of isolates in potato plant by germination per cent.

Table 9 Effect of endophytic bacterial isolates on plant height at 30,45 and 90 DAS.

S. No.	Isolates No.	30 DAS	45 DAS	90 DAS
1	T1	18.39 ^{b*}	40.23 ^b	62.84 ^b
2	T2	16.28 ^{ef}	35.98 ^c	49.28 ^e
3	T3	15 ^g	32.21 ^d	51.27 ^d
4	T4	16.05 ^f	36.51 ^c	44.58 ^f
5	T5	17.25 ^{cd}	32.85 ^d	50.28 ^{de}
6	T6	16.5 ^e	35.55 ^c	44.27 ^f
7	T7	17.11 ^d	37.69 ^c	41.22 ^g
8	T8	16 ^f	33.74 ^d	38.69 ^h
9	T9	17.54 ^c	38.62 ^b	49.26 ^e
10	T10	18.27 ^b	39.36 ^b	58.05 ^c
11	T11	19.31 ^a	46.25 ^a	67.22 ^a
	CV%	1.59	2.38	2.16
	SEm	0.073	0.81	1.18

* Means with the same letter are not significantly different at ($p = 0.05$).

by Mumtaz et al. (2017) where maize yield was enhanced with inoculation of maize seed with zinc solubilizing bacteria.

P, K, Zinc contents in Shoots and tubers of inoculated and un-inoculated plants

A significant increase in P, K, and zinc content of shoots and tubers was

observed for the plants inoculated with all the endophytic isolates compared to the inoculated control. The highest amounts of P, K, and zinc were detected in shoots of treatment 11, which had 7.21, 45.29, and 0.041 mg/g of P, K, and Zn, respectively. The P, K, and Zn content of tubers was highest

Table 10 Effect of endophytic isolates on concentration of P, K Zn content of shoots.

S. No.	Isolates No.	Phosphorus concentration in shoots (mg g ⁻¹)	Potassium concentration in shoots (mg g ⁻¹)	Zinc Concentration in shoots (mg g ⁻¹)
1	T1	5.35 ^{d*}	34.67 ^c	0.017 ^k
2	T2	2.95 ^h	14.35 ⁱ	0.026 ^e
3	T3	5.26 ^{de}	18.07 ^g	0.019 ^j
4	T4	3.06 ^h	29.33 ^e	0.022 ^g
5	T5	4.05 ^f	15.47 ^h	0.038 ^b
6	T6	5.17 ^e	22.98 ^f	0.018 ⁱ
7	T7	4.18 ^f	32.52 ^d	0.020 ^h
8	T8	3.87 ^g	30.26 ^e	0.031 ^d
9	T9	6.32 ^b	35.61 ^c	0.025 ^f
10	T10	5.66 ^c	38.19 ^b	0.032 ^c
11	T11	7.21 ^a	45.29 ^a	0.041 ^a
	CV %	1.95	2.70	4.19
	S.Em	0.009	0.6	2.47

*Means with the same letter are not significantly different at ($p = 0.05$).

for T11 (Table 10). Plants inoculated with strains PSB1 and PSB2 showed an increase in tubers and shoot P content as compared to un-inoculated controls. Plants inoculated with strains KSB1 and KSB2 showed an increase in tubers and shoot K content as compared to un-inoculated controls. Plants inoculated with strains ZSB1 and ZSB2 showed an increase in tuber and shoot Zn content as compared to un-inoculated controls (Table 11). These results indicate the efficacy of these PGPR strains in enhancing the bioavailability of P, K, and zinc and mobilising it toward tubers. Ghanbari et al (2022) demonstrated that the application of potassium, phosphorus, and iron-solubilizing bacteria considerably boosted the growth parameters of potato plants as well as their absorption of phosphorus, potassium, iron, and zinc. *Pseudomonas frederiksbergensis* to be the most potent bacterium in this investigation for most plant growth indices and nutrient absorption.

Table 11 Effect of endophytic isolates on concentration of P, K, Zn content of tubers.

S. No.	Isolates No.	Phosphorus concentration in tubers (mg g ⁻¹)	Potassium concentration in tubers (mg g ⁻¹)	Zinc Concentration in tubers (mg g ⁻¹)
1	T1	3.15 ^{ae}	36.21 ^b	0.008 ^g
2	T2	2.00 ^h	15.32 ^j	0.010 ^e
3	T3	3.81 ^c	20.05 ⁱ	0.001 ⁱ
4	T4	2.08 ^h	32.48 ^d	0.008 ^g
5	T5	2.35 ^g	13.61 ^k	0.017 ^b
6	T6	3.63 ^d	21.82 ^g	0.002 ^h
7	T7	3.09 ^e	34.27 ^c	0.009 ^f
8	T8	2.84 ^f	21.09 ^h	0.011 ^f
9	T9	3.96 ^b	31.36 ^e	0.016 ^d
10	T10	3.71 ^{cd}	28.29 ^f	0.028 ^c
11	T11	4.18 ^a	45.08 ^a	0.035 ^a
	CV %	2.60	1.78	6.52
	S.Em	0.007	0.23	2.36

*Means with the same letter are not significantly different at (p < 0.05).

Inoculation efficacy of Isolates by fresh biomass, dry biomass, and tuber weight of Potato Plants at harvesting

Application of 75% NPK +Zn with endophytic isolates improves fresh biomass and tuber weight compared to uninoculated treatment (Table 12). The above results can be directly correlated with results of endophytic bacterial isolates from potato efficiency as well as in vitro studies of PGP traits, which may have a possible role in potato growth enhancement showing multiple PGP traits possession ultimately leading to enhanced plant growth. Growth hormone (IAA) and the availability of P, K, and Zn by inoculating endophytic isolates had a positive effect on germination rate, root growth, and shoot growth. Similarly, Khalil (2009) reported that single inoculation with bacteria, *P. putida*, *A. brasilense*, and *B. megaterium*, had a significant positive effect on shoot dry matter and also significantly increased root dry matter of potato plants. Kannapiran and Ramkumar (2011) reported that *A. chroococcum*, *A.*

Table 12 Effect of endophytic bacterial isolates on fresh biomass, dry biomass and tuber weight of potato plants.

S. No.	Isolates No.	Fresh biomass (g)	Dry biomass (g)	Tuber weight (g)
1	T1	34.31 ^{ce}	24.15 ^c	154.39 ^c
2	T2	26.31 ^g	18.36 ^h	125.41 ^g
3	T3	34.27 ^c	25.08 ^b	139.36 ^f
4	T4	32.09 ^e	21.58 ^{de}	147.62 ^e
5	T5	32.59 ^{de}	20.19 ^f	152.78 ^d
6	T6	33.25 ^{cd}	24.16 ^c	150.20 ^{de}
7	T7	29.15 ^f	19.02 ^g	142.14 ^f
8	T8	30.11 ^f	22.15 ^d	140.34 ^f
9	T9	37.48 ^b	21.32 ^e	165.69 ^b
10	T10	35.61 ^c	24.00 ^c	157.95 ^c
11	T11	39.26 ^a	30.69 ^a	179.65 ^a
	CV %	2.28	1.84	6.52
	S. Em	0.58	0.18	2.36

* Means with the same letter are not significantly different at (p < 0.05)

beijerinckii, *P. aeruginosa*, and *Bacillus spp.* enhanced shoot length, root length, and total dry mass as compared to control in black gram.

CONCLUSION

Endophytic bacteria are plant-beneficial bacteria that exist within plants and can enhance plant growth under normal and adverse conditions. Being a vegetatively propagated crop, the potato continuously perpetuates a large number of microorganisms in its tuber as well as in its roots. We isolated P, K, Zn solubilizer and siderophore-producing bacteria from tubers and roots of potatoes. Endophytic bacterial isolates were capable of surviving high NaCl concentrations and a wide pH range. In vitro phosphate, potassium, zinc solubilization, siderophore production, and potato plant growth stimulation were observed by all isolates compared with the uninoculated control. Seed tuber bacterization with endophytic bacterial isolates in conjunction with 3/4 RD of NPK + Zn can substitute 1/4 of fertilizer requirement of potato.

CONFLICTS OF INTEREST

The authors confirm that this manuscript has no conflicts of interest

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