Identification and Characterization of *Aeromonas* sp. KBK3 as a Multifunctional Plant Growth-Promoting Rhizobacterium from Banana

Govinda Pathak[1], Sandip Ghimire[1], Gyanu Raj Pandey[2], Junxi Shi[3], Manish Rijal[1], Durga Karki[1], Janardan Lamichhane[1]

[1] Department of Biotechnology, Kathmandu University, Kavre, Nepal
[2] Shubham Biotech Nepal Private Limited, Bharatpur, Chitwan, Nepal
[3] Food Safety Detection Key Laboratory of Sichuan Province, Chengdu, China

***CORRESPONDING AUTHOR:**
Janardan Lamichhane
Email: ljanardan@ku.edu.np

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ABSTRACT

In sustainable agriculture, plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria, that enhance nutrient availability and improve plant resilience to stress. This study was conducted to isolate and characterize PGPR from the banana (*Musa* sp.) rhizosphere. An efficient strain, identified as *Aeromonas* sp. KBK3 via biochemical tests and 16S rRNA gene sequencing, demonstrated significant plant growth-promoting traits. It showed noteworthy potassium solubilization index of 3.77 ± 0.27 and produced 11.4 ± 0.28 ppm of indole-3-acetic acid (IAA). The strain showed optimal growth at 35°C, pH 7, 0.5% NaCl, and tolerated 30% osmotic stress. Its PGPR traits include potassium and phosphorus solubilization, IAA production, extracellular enzymes such as cellulase and pectinase production, and tolerance to 30% polyethylene glycol (PEG). In addition, the strain didn't show any phytotoxic effects towards spinach seed germination. Therefore, *Aeromonas* sp. KBK3 is a promising biofertilizer to increase nutrient availability and crop productivity. However, it must be validated through greenhouse and field trials for its agronomic performance.

Keywords: Plant growth promoting rhizobacteria (PGPR), Potassium solubilization, Indole-3-acetic acid (IAA), Phosphate solubilization, germination, biofertilizer

1. INTRODUCTION

Global food security is increasingly threatened by a confluence of factors: steady population growth, the shrinkage of cultivable land, and the escalating challenges of climate change (Daniel *et al.* 2022; Alzate Zuluaga *et al.* 2024). To meet rising demands, agricultural systems have become dependent on synthetic inputs, yet their use poses serious adverse effects on both environmental integrity and human health (Saeed *et al.*

2021). This unsustainable paradigm underscores the critical necessity of developing alternative production systems that are both sustainable and eco-friendly. A promising approach involves harnessing beneficial microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), to enhance crop productivity (Saeed *et al.* 2021; Yang *et al*. 2024).

Characterized by complex plant-soil-microbe interactions, the rhizosphere constitutes a critical hotspot for microbial processes (Basu *et al.* 2021; Chandran *et al.* 2021; Solomon *et al.* 2024; Wang *et al.* 2024). Through the release of complex mixtures of photosynthates, known as rhizodeposits, plants exude chemical signals that selectively recruit and shape the surrounding microbial community (De Andrade *et al.* 2023; Solomon *et al.* 2024). This dynamic interplay directly impacts plant physiology and health. Among the key beneficiaries and mediators of this relationship are PGPR, a heterogeneous group of bacteria that efficiently colonize the rhizoplane and contribute to plant fitness (Basu *et al.* 2021; Yang *et al.* 2024).

The banana (*Musa* sp.) rhizosphere represents a prime niche for prospecting novel PGPR, a promise derived from its nutrient-rich root exudates and stable physicochemical conditions. These attributes support microbial communities that perform essential functions, directly enhancing plant growth, nutrient acquisition, and overall productivity (Yang *et al.* 2021; Muzami *et al.* 2025). Dominant among these communities are beneficial phyla such as Proteobacteria, Actinobacteria and Cyanobacteria (Muzami *et al.* 2025). PGPR strains isolated from this environment often possess multiple plant-beneficial traits, including the production of phytohormones like indole-3-acetic acid (IAA), the solubilization of essential nutrients (e.g., phosphate, potassium, zinc), nitrogen fixation, chlorophyll biosynthesis and biocontrol activity against pathogens (KC *et al.* 2020). Consistent with this functional diversity, several bacterial genera are recurrently identified as effective PGPR in banana systems, including *Bacillus, Pseudomonas, Enterobacter, Kosakonia, Paenibacillus,* and *Streptomyces.* Specific species, such as *Bacillus subtilis, Pseudomonas putida,* and *Streptomyces aureoverticillatus*, have been highlighted for their exceptional PGPR activity and efficacy as biocontrol agents (Posada *et al.* 2016; Yang *et al.* 2021; Tatung and Deb 2023).

This study aims to isolate and characterize native PGPR from the banana rhizosphere to identify highly efficient, ecologically adapted strains. The goal is to develop these strains into microbial inoculants that enhance crop vigor, improve root architecture, strengthen nutrient acquisition, and promote disease suppression. Ultimately, this approach seeks to foster a resilient root microbiome, thereby contributing to agroecosystem resilience and food security.

2. MATERIALS AND METHODS

2.1 Materials and Equipment

All chemicals, growth media, and reagents were of microbiological or molecular biology grade. General chemicals and growth media were procured from HiMedia and Fisher Scientific, while molecular biology reagents, buffers, and enzymes were obtained from Thermo Scientific, Cleaver Scientific, SRL, and New England Biolabs. Specific kits, including the Genomic DNA extraction kit (Genes2me, India) and Hi-Carbo kit (HiMedia), were used as per the manufacturers' instructions. PCR primers were synthesized by Barcode Biosciences.

Laboratory consumables included glassware from Borosil and plasticware from Tarson. The following equipment was used: a Labomed Lx 400 microscope for microscopic characterization; a Lab Companion IB-01-65L incubator for culture growth; a Nuve FN120 oven for drying; and a Shimadzu UV-1800 spectrophotometer for absorbance measurements. Centrifugation was performed using MPW-260R centrifuge for DNA extraction and a Hermle Z-336 model for other processes. PCR amplification was carried out in a Bio-Rad T100TM Thermal Cycler, and DNA fragments were visualized using a GenoSens 2100 gel documentation system.

2.2 Sample collection, Isolation and Confirmation of Potassium Solubilizing Bacteria (KSB)

Rhizospheric soil samples were collected from a banana field in Khatrithowk, Sindhupalchowk, Nepal (Elevation: 1028 m; Coordinates: 27°41′54″ N and 085°43′53″ E; see Fig. 1) using a Garmin eTrex 10 handheld GPS receiver. For microbial isolation, 10 g of soil was weighed using a BEL Engineering M124A balance and serially diluted in 90 mL of sterile distilled water. Aliquots (1 mL) from appropriate dilution were plated via the pour plate method onto sterilized Aleksandrow agar. The plates were incubated at 30 ºC for 24 hours. Distinct colonies were purified by

repeated streaking on nutrient agar. The pure isolated colony was spot inoculated into an Aleksandrow agar plate and incubated at 28 °C for 4 days. The potassium solubilization index (KSI) was calculated using the formula(Sun *et al.* 2020; Yaghoubi Khanghahi *et al.* 2021):

$$KSI = \frac{\text{diameter of solubilization halo} + \text{colony diameter}}{\text{Colony diameter}}$$

Pure cultures were maintained on Nutrient agar slants for short-term storage and preserved long-term at -80 °C in cryovials containing the respective basal medium supplemented with 20% glycerol (Cappuccino and Welsh 2019).

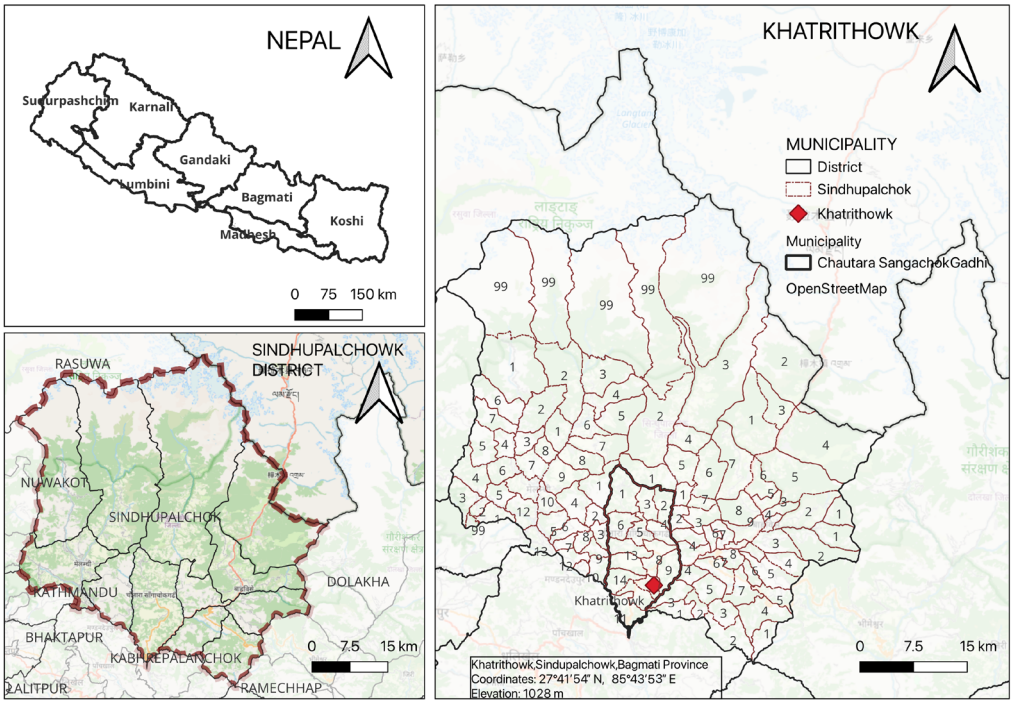


Fig 1: *Administrative map of Khatrithowk (Ward No. 10, Chautara Sangachokgadhi Municipality) showing ward boundaries and the sampled site (red diamond).*

2.3 Identification of Selected Bacterial Strain

2.3.1 Molecular Identification

Genomic DNA (gDNA) was purified from the isolated organism by bacterial DNA extraction kit. About 2 ml of the isolate, cultured in 5 ml nutrient broth at 30 ºC for 24 hours, was centrifuged at 15000g for a minute. The pellet was processed as per manufacturer's protocol.

The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') /1492R (5'-TACGGYTACCTTGTTACGACTT -3') primers (22) and sequenced by 785F (5'-GGATTAGATACCCTGGTA-3') /907R (5'-CCGTCAATTCMTTTRAGTTT- 3') at Macrogen Inc., South Korea.

Raw sequences were trimmed and assembled using Codon Code Aligner v 12.0.1 (CodonCode) and the consensus sequence was subjected to BLASTN and the database—rRNA/ITS database was selected in NCBI. Sixteen highly similar sequences (based on BLAST result) along with our sequence were taken in FASTA format for phylogenetic analysis. A maximum likelihood phylogenetic tree was constructed in MEGA12 (Tamura et al. 2021) via the Tamura-Nei model (Tamura and Masatoshi Nei 1993), with nodal support assessed via 2,000 bootstrap replicates (Felsenstein 1985).

2.3.2 Morphological and Biochemical

Characterization

The isolated strain was cultured on nutrient agar and incubated at 30 °C for 24 hours to study its colony morphology, documenting characteristics including shape, size, pigmentation, elevation, margin, opacity, surface texture, and any change in medium color. Micromorphological analysis was performed via Gram staining using a HiMedia Kit following established methods (Cheesbrough 2006), with subsequent observation under a light microscope at 1000X magnification. Biochemical characterization included standard tests for methyl red, Voges-Proskauer, oxidase, catalase, and citrate utilization (Cappuccino and Welsh 2019). Carbohydrate utilization profiles were determined using a HiCarbo kit according to the manufacturer's instructions, and all morphological and biochemical data were interpreted with reference to Bergey's Manual of Systematic Bacteriology (Martin-Carnahan and Joseph 2015).

2.4 Growth under Different Abiotic Stress Conditions

2.4.1 Temperature

The optimal growth temperature was determined by inoculating 10 mL of nutrient broth with a loopful of an overnight culture and incubating at 25, 30, 35 and 40ºC. After 24 hours of incubation, bacterial growth was quantified by measuring optical density at 600 nm using UV-Vis spectrophotometer (Agunbiade *et al.* 2024).

2.4.2 Salt Concentrations

Salt tolerance was assessed in nutrient broth supplemented with varying concentrations of NaCl (0.5%, 5.5%, and 10.5% w/v). The medium was inoculated with a loopful of an overnight bacterial culture and incubated at 30ºC for 24 hours. Growth under these saline conditions was measured as OD_{600} (Agunbiade *et al.* 2024).

2.4.3 pH

To evaluate growth at different pH levels, the bacterium was inoculated into 10 mL of nutrient broth adjusted to acidic (pH 4), neutral (pH 7), and alkaline (pH 10) conditions using appropriate buffers. The cultures were incubated at 30ºC for 24 hours, and growth was determined spectrophotometrically at 600 nm (Agunbiade *et al.* 2024).

2.4.4 Drought

Drought stress was simulated using polyethylene glycol (PEG 400, SRL-25322-68-3). A loopful of bacterial culture was used to inoculate nutrient broth supplemented with 0%, 15%, and 30% (v/v) PEG. The cultures were incubated at 30°C for 24 hours, and bacterial growth was assessed by measuring the OD_{600} (Sandhya *et al.* 2009; Agunbiade *et al.* 2024; Noureen *et al*. 2024; Rafedzi *et al*. 2024).

2.5 Plant Growth Promoting Traits

2.5.1 Indole-Acetic Acid (IAA) Production

IAA production was quantified following the method of Loper and Schroth. A pure bacterial isolate was inoculated into DEV tryptophan broth and incubated at 30 ºC for 72 hours. The bacterial suspension was then centrifuged at 5000 g for 20 minutes at 4 ºC. Subsequently, 2mL of the cell-free supernatant was mixed with 4 mL of Salkowski's reagent and a few drops of orthophosphoric acid (H_3PO_4). The development of pink color indicated IAA production. The IAA concentration was determined by measuring the absorbance at 530 nm and comparing it to a standard IAA calibration curve (AlAli *et al.* 2021; Agunbiade *et al.* 2024).

2.5.2 HCN Production test

The potential for HCN production was assessed using nutrient agar supplemented with glycine (4.4 g/L). Bacterial cultures were streaked onto the agar, and a filter paper soaked in a picrate solution (0.5% picric acid and 1% Na_2CO_3) was attached to the underside of the plate lid. The plates were sealed with parafilm to prevent desiccation and incubated at 30°C for 48 hours. A color change of the filter paper from yellow to reddish-brown was considered a positive result (Agunbiade *et al.* 2024).

2.5.3 Phosphate solubilization

The ability to solubilize inorganic phosphate was tested on Pikovskaya agar medium. The agar plates were spot-inoculated with an overnight bacterial culture and incubated at 30°C for 4 days. The formation of a clear halo zone around the bacterial colony was indicative of phosphate solubilization (Wan *et al.* 2020).

2.6 Hydrolytic Enzyme Production

2.6.1 Protease

Protease production was assessed using skimmed milk agar. Plates were inoculated in a zigzag pattern with the bacterial culture and incubated at 30°C for 24 hours. The formation of a clear halo zone around the bacterial growth, indicating casein hydrolysis, was recorded as a positive result (Lakshmi 2014; Marathe *et al*. 2018).

2.6.2 Amylase

Amylase activity was detected on nutrient agar supplemented with 1% (w/v) starch. After the bacterial strain was streaked in zigzag pattern onto the medium and incubated at 30°C for 24 hours, the plates were flooded with an iodine solution. A clear zone surrounding the bacterial growth against a dark blue background confirmed starch hydrolysis and a positive result (AlAli *et al.* 2021).

2.6.3 Cellulase

Cellulase production was evaluated on nutrient agar containing 1% (w/v) carboxymethyl cellulose (CMC). The medium was streaked with bacterial strain and incubated at 30°C for 24 hours. The plates were then flooded with a 0.1% Congo red and washed with 1M NaCl. The formation of a clean zone around the colony was recorded as a positive result (Gautam *et al.* 2012).

2.6.4 Pectinase

Nutrient agar incorporated with 1% pectin was used for the isolation and screening of pectinolytic bacteria. The initial pH of medium was adjusted to 4.5. This medium was sterilized and distributed aseptically in Petri dishes. Isolates were streaked into this media and incubated at 30°C for 24 hours. For primary screening, plates were stained with 50 mM iodine for result. A clear halo zone around the colonies indicates the pectinolytic activity of bacteria (Khairnar *et al*. 2009; Raju and Divakar 2013).

2.6.5 Chitinase

For screening of chitinase producing bacteria, the nutrient agar medium amended with 1% (w/v) colloidal chitin was used. The colonies showing clearance zones, after 24 hours upon incubating at 30°C, on a creamish background were considered as chitinase-producing bacteria (Saima *et al.* 2013).

2.7 Seed Germination Test

A seed vigour assay was conducted under laboratory conditions to evaluate the plant growth-promoting potential of selected bacterial isolates on broad leaf mustard (*Brassica juncea* var. Khumal Chaudapat). Indigenous seeds were procured from the Nepal Agricultural Research Council (NARC), Khumaltar.

Seeds were surface-sterilized by immersion in 70% (v/v) ethanol for one minute, followed by rinsing with sterile distilled water. Subsequently, seeds were treated with a 5% (v/v) sodium hypochlorite solution for 4-5 minutes and thoroughly rinsed 4-6 times with sterile distilled water. A bacterial suspension was prepared by harvesting a 24-hour-old nutrient broth culture via centrifugation and resuspending the cells in 0.85% saline to an optical density (OD_{600}) of 1. The surface-sterilized seeds were then immersed in this bacterial suspension for two hours. Following inoculation, the seeds were blotted dry on sterile filter paper (Sartorious, 393) for 12 hours. For the germination test, one hundred treated seeds were placed aseptically in 90 mm petri plates, with three replicates per treatment. The plates were maintained in an incubator at 25°C, regularly watered, and monitored daily for germination over eight days. A control treatment consisted of surface-sterilized seeds treated with 0.85% saline solution without bacteria. The number of germinated seeds was counted every day after incubation, and each spinach seed was adjudged as germinated if the radicle has emerged. Post priming radicle and plumule (early seedling) lengths were measured following standard assessment methods used for spinach, with measurements taken on day 8 after sowing on petri plate (Chen and Arora 2011; Wang *et al.* 2019).

2.8 Statistical Analysis

Statistical analyses were carried out in OriginPro 2024. A one-way analysis of variance (ANOVA) was used to determine the significance of differences among treatment groups for quantitative data. Where ANOVA indicated a significant effect ($p < 0.05$), Tukey's post-hoc test was applied for multiple comparisons. Specifically, for the seed germination assay, a two-tailed, paired-sample t-test was employed to compare the means of the treatment and control groups, with the null hypothesis of no difference between means. All experiments in this study were conducted in triplicate ($n = 3$), and the mean values with standard error were calculated accordingly.

3. RESULT

3.1 Potassium Solubilization Index

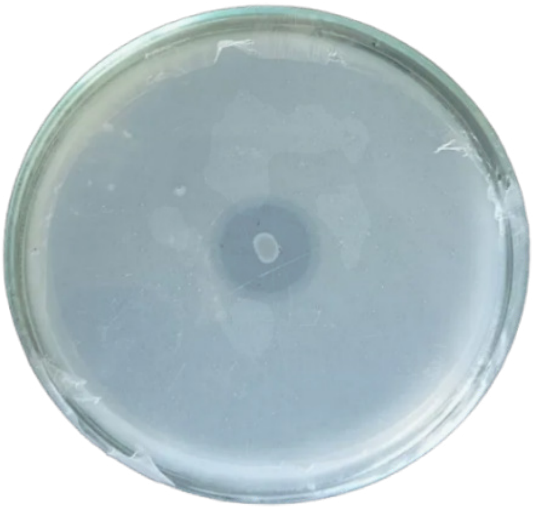


Fig 2: *Potassium solubilization index (KSI) (3.77± 0.27) of the KBK3. Clear halo zones around colonies on potassium-bearing medium indicate solubilization*

Initial screening of bacterial isolate from the banana rhizosphere on Alexandrow agar led to the identification of a promising potassium-solubilizing bacterium (KSB), strain KBK3. The isolate demonstrated a high potassium solubilization index (KSI) of 3.77 ± 0.27 after a 4-day incubation period (Fig. 2).

3.2 Identification of Selected Bacterial Strain

3.2.1 Molecular Characterization of the isolate

A maximum-likelihood phylogenetic tree (Fig. 3), based on 16S rRNA gene sequences, was constructed to determine the taxonomic position of the environmental isolate KBK3 within the genus *Aeromonas*. The tree revealed that KBK3 forms a distinct branch within a well-supported clade containing *Aeromonas dhakensis* strain BLW14 and *Aeromonas* media strain BCPP1. Despite this close association, measurable genetic distances (0.01 – 0.02) separate KBK3 from its nearest neighbor, *A. dhakensis* BLW14, indicating a degree of sequence divergence. This isolate consistently clustered within the broader *Aeromonas* lineage, with high bootstrap support at major nodes, confirming its unambiguous placement within this genus while highlighting its phylogenetic distinctiveness from other closely related species and strains included in the analysis.

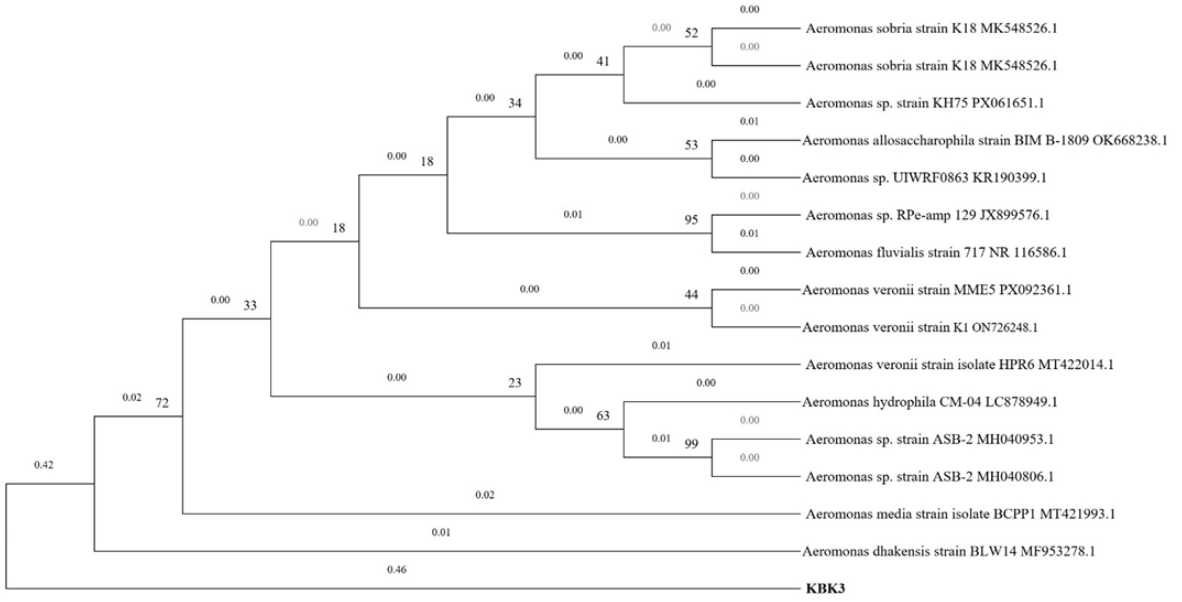


Fig 3: *Maximum likelihood phylogenetic tree of the isolate based on 16S rRNA sequence data (Tamura-Nei model). The tree is drawn with branch lengths (shown next to the branches) computed using the maximum likelihood method and measured in the number of substitutions per site. Bootstrap values (2000 replicates) were shown at nodes; branches with <50% support were collapsed.*

3.2.2 Phenotypic descriptions of the isolate

The isolate KBK3 exhibited phenotypic characteristics consistent with members of the genus *Aeromonas*. Colonies on nutrient agar medium were circular, convex, entire-margined, creamy-white, opaque, and displayed a smooth, sticky texture. Biochemically, KBK3 was positive for oxidase, catalase, and citrate utilization, and was motile. It produced acid and gas from glucose, sucrose, and lactose on triple sugar iron agar, but did not produce H_2S. The isolate demonstrated as extensive carbohydrate fermentation profile (Table 1), utilizing a wide range of sugars including lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, mannose, inulin, sodium gluconate, glycerol, salicin, dulcitol, inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, α-methyl-D-glucoside, rhamnose, cellobiose, melezitose, α-methyl-D-mannoside, xylitol, ONPG (O-nitrophenyl-β-D-galactopyranoside), esculin, D-arabinose, citrate, malonate, and sorbose, but was negative for L-arabinose fermentation. On blood agar, KBK3 displayed γ-hemolysis.

Table 1: Phenotypic characterization of bacterial isolate comparison with closely related species based on BLAST result

Test	KBK3	A. *hydrophila* (Abbott *et al.* 2003; Abulhamd 2009; Ahangarzadeh *et al.* 2022)
Colony morphology		
Size(mm)	1.67	
Gram staining	-	-
Elevation	Convex	
Margin	Entire	
Colony form	Circular	
Pigmentation	Creamy white	
Opacity	Opaque	

Surface appearance	Smooth	
Texture	Sticky	
Oxidase	+	+
Catalase	+	+
H_2S	-	-
Motile	+	+
Methyl-red	-	+
Voges-Proskauer	-	+/-
Blood agar	γ Hemolysis	β hemolytic
Sugar Utilization		
Lactose	+	-/+
Xylose	+	-
Maltose	+	+
Fructose	+	
Dextrose	+	+
Galactose	+	
Raffinose	+	-
Trehalose	+	+
Melibiose	+	-
Sucrose	+	+
L-Arabinose	-	+
Mannose	+	+
Inulin	+	-
Sodium gluconate	+	+/-
Glycerol	+	+
Salicin	+	+/-
Dulcitol	+	-
Inositol	+	-
Sorbitol	+	-
Mannitol	+	+
Adonitol	+	-
Arabitol	+	-
Erythritol	+	-
α-Methyl-D-glucoside	+	+/-
Rhamnose	+	-/+
Cellobiose	+	-
Melezitose	+	
α-Methyl-D-Mannoside	+	
Xylitol	+	-
ONPG	+	+

Esculin	+	+
D-Arabinose	+	+
Citrate	+	+
Malonate	+	-
Sorbose	+	

3.3 Growth under different abiotic stress

3.3.1 Temperature

The isolate exhibited optimal growth at 35°C, achieving an OD_{600} of 0.645 ± 0.007 after 24 hours. Growth was significantly reduced at 30°C (0.57 ± 0.00153), 25°C (0.352 ± 0.00379), and 40°C (0.049 ± 0.00493), as determined by one-way ANOVA and Tukey's test ($p < 0.05$) (Fig. 4a).

3.3.2 Salt

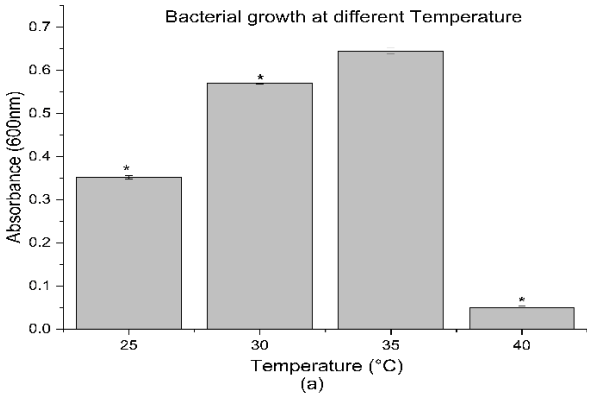
Bacterial growth under saline conditions was evaluated at concentrations of 0.5%, 5.5%, and 10.5% NaCl by measuring optical density at 600 nm after 24 hours. The isolate showed optimal growth at 0.5% NaCl (0.49733 ± 0.0229). Statistical analysis (Tukey's test, $p < 0.05$) revealed that growth at this concentration was significantly higher than at 5.5% (0.19167 ± 0.00416) and 10.5% (0.07067 ± 0.00404) NaCl (Fig. 4b).

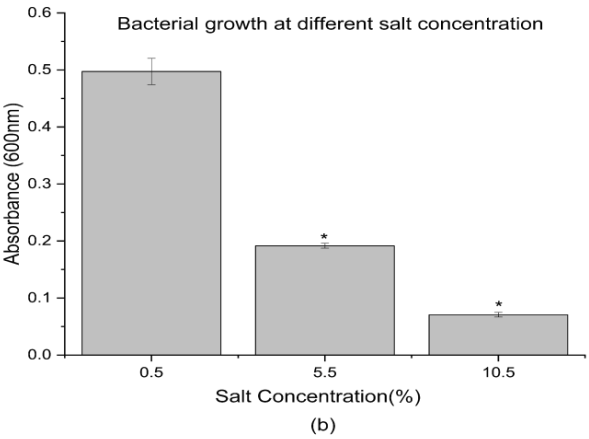
3.3.3 pH

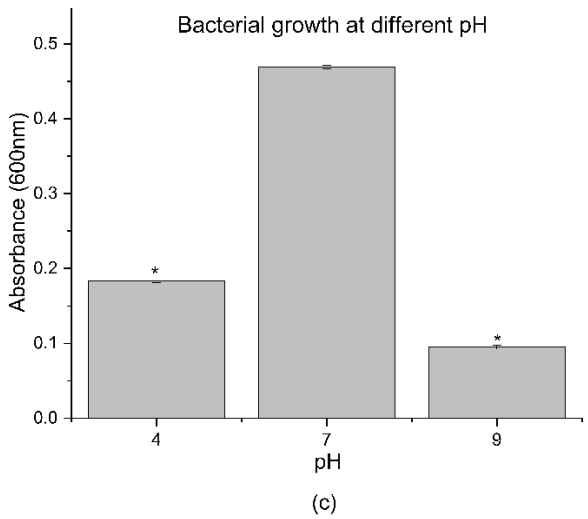
The effect of pH on bacterial growth was assessed at pH 4, 7, and 9 by measuring optical density at 600 nm after 24 hours. Optimal growth occurred at pH 7 (0.46933 ± 0.00233). Statistical analysis revealed this growth was significantly higher than at pH 4 (0.18333 ± 0.00203) and pH 9 (0.095 ± 0.00252), according to Tukey's post-hoc test ($p < 0.05$) (Fig. 4c).

3.3.4 Drought

To assess drought tolerance, bacterial growth was measured in media supplemented with 0%, 15%, and 30% PEG. After 24 hours, optical density at 600 nm revealed a significant, concentration-dependent inhibition. While significantly higher ($p < 0.05$) growth occurred in the absence of PEG (0.553 ± 0.00265), it was markedly reduced at 15% PEG (0.21933 ± 0.00521) and 30% PEG (0.10833 ± 0.00328) (Fig. 4d).







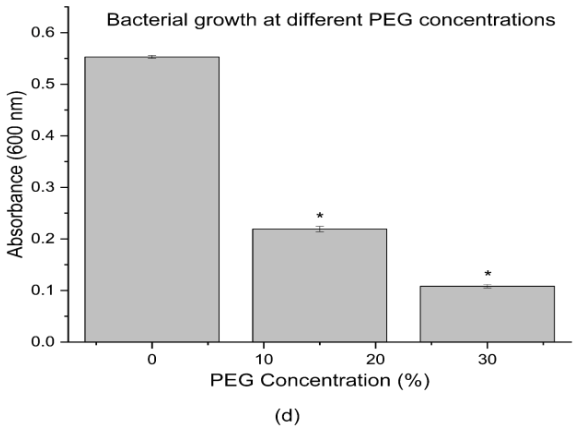


Fig 4: *Growth of KBK3 in nutrient broth under (a) varying temperature; (b) varying concentration of salt (%); (c) varying PH and (d) varying concentration of PEG (%). * Significantly higher than the compared groups (α = 0.05).*

3.4 Plant Growth Promoting Traits

3.4.1 Indole Acetic acid (IAA) production

This study quantified indole-3-acetic acid (IAA) production by the bacterial isolate. A standard calibration curve (y= 0.01682x + 0.04027; Pearson's r = 0.99551) (Fig. 5) was used to determine concentrations. Following 72 hours of incubation in DEV tryptophan broth, isolate KBK3 demonstrated the ability to synthesize IAA, producing 11.4 ± 0.28 ppm.

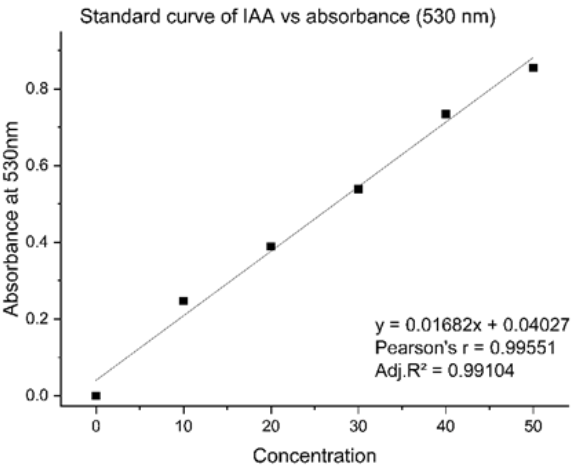


Fig 5: *Standard curve of IAA concentration vs absorbance at 530 nm*

3.4.2 Determination of other plant growth promoting traits

Assessment of key plant growth-promoting traits revealed that strain KBK3 solubilized phosphate (Table 2). However, it did not exhibit nitrogen-fixing activity or produce hydrogen cyanide (HCN).

Table 2: Plant growth promoting traits exhibited by KBK3

Plant growth traits	KBK3
Nitrogen fixation	-
Phosphate solubilization	+
HCN produced	-

3.5 Hydrolytic enzymes production

KBK3 demonstrated a selective enzymatic profile, positive for cellulase and pectinase production but negative for chitinase, protease, and amylase (Table 3).

Table 3: Hydrolytic enzymes produced by KBK3

Enzymes produced	KBK3
Cellulase	+
Chitinase	-
Proteinase	-
Amylase	-
Pectinase	+

3.6 Seed Germination

Seed germination and early seedling growth parameters were analyzed using a paired two-sample t-test. While PGPR-treated seeds showed no significant difference in germination rate compared to the control (28 ± 1.15 vs. 29.33 ± 0.88 seeds, p = 0.27) and exhibited no phytotoxic effects, treated seedlings demonstrated non-significant increase in total length (5.64 ± 0.25 cm vs. 5.12 ± 0.24 cm), radicle length (3.21 ± 0.22 cm vs. 2.77 ± 0.20 cm), and plumule length (2.43 ± 0.12 cm vs. 2.34 ± 0.10 cm). These results indicate that, although statistically non-significant under the tested conditions, the PGPR inoculation was safe and showed a positive trend toward enhancing early seedling vigor.

4. DISCUSSION

The banana rhizosphere isolate KBK3 demonstrated rapid potassium solubilization, achieving a high

solubilization index (KSI) of 3.77 ± 0.27 within four days. This efficiency is significant when compared to the broader spectrum of potassium-solubilizing microorganisms (KSMs), which include genera such as *Bacillus, Paenibacillus, Pseudomonas, Burkholderia, Enterobacter, Aspergillus, Penicillium,* and several *actinomycetes* that mobilize insoluble soil potassium through organic acid secretion such as tartaric acid, citric acid, and oxalic acid (Olaniyan *et al.* 2022; Sharma *et al*. 2024).

Our isolate's performance places it among the more efficient and faster-acting potassium-solubilizing bacteria (KSB) reported. It exceeds the KSI of several moderate-efficiency KSB, such as *Metabacillus indicus* (KSI ~ 3 after 7 days) (Fatema *et al*. 2024), halotolerant species like *Halomonas and Oceanobacillus* (KSI 2.33-2.96 after 5 days) (Reang *et al.* 2022), and other *Aeromonas* strains (KSI 2.25-2.98 after 5 days) (Antonelli *et al.* 2025). It also compares favorably to *Serratia* sp. Bg22c (KSI 2.50 after 2 days) (Bouizgarne *et al.* 2023) and high-performing actinomycetes that require longer incubation, such as *Streptomyces anulatus* (KSI 3.17 after 11 days) (Boubekri *et al.* 2021). While some isolates from specific niches report higher indices (e.g., KSI up to 6.70 after 7 days) (Saheewala *et al.* 2023), they generally require nearly twice the incubation time. Similarly, other studies report indices in the range of 1.57-5.67 after 7 days (Nawaz et al. 2023), 1.79 after 7 days at low temperature (Yan *et al.* 2024), and more than 2 after 8 days (Raji and Thangavelu 2021). Thus, the combination of a high KSI and a short incubation period underscores the notable solubilization capacity of KBK3. This trait, central to its biofertilizer potential, can enhance plant-available potassium and reduce dependency on chemical inputs, offering a promising sustainable alternative for nutrient management in agriculture.

Based on the integrated phylogenetic and phenotypic features such as gram negative, motile, oxidase & catalase positive, H_2S negative, utilization of maltose, dextrose, trehalose, mannitol, ONPG and esculin, isolate KBK3 can be confidently assigned to the genus *Aeromonas*. The phylogenetic reconstruction placed KBK3 within a well-supported clade of *Aeromonas* species, most closely related to *Aeromonas dhakensis*, yet the observed genetic distances and notable biochemical variations, such as its broad sugar fermentation profile in raffinose, melibiose, L-arabinose, inulin, dulcitol, inositol, sorbitol, adonitol, arabitol, erythritol, cellobiose, xylitol, & malonate, and non-hemolytic activity, suggest that KBK3 may represent a distinct strain within the genus (Abbott *et al.* 2003; Martin-Carnahan & Joseph 2015; Abo-Shama *et al.* 2025). These discrepancies, coupled with the high metabolic versatility observed in biochemical assays, support a conservative classification as *Aeromonas* sp. KBK3, reflecting its clear genus-level affiliation while acknowledging sufficient divergence to preclude reliable species-level identification without further genomic characterization.

The indole-3-acetic acid (IAA) production of *Aeromonas* sp. KBK3 was quantified at 11.4 ± 0.28 ppm (µg/mL) after 72 hours of incubation. This output situates the strain within as intermediate range of phytohormone synthesis, a level often associated with the beneficial modulation of root architecture by plant growth-promoting rhizobacteria (PGPR). When compared to other PGPR, KBK3's production exceeds that of weaker producers, such as *Bacillus zanthoxyli* strain GGS1 (1.5 ppm after 9 hours) (Kim *et al.* 2024) and *Stenotrophomonas* sp. strain C1 and C10 (8.94 ppm and 2.68 ppm after 5 days, respectively) (Abd-Alla *et al.* 2024). Conversely, it is substantially lower than that of highly efficient producers like *Klebsiella* sp. CK-40 and *Bacillus cereus* M28, which can yield over 41.06 ppm and 378.44 ppm, respectively (Ganesh *et al*. 2024; Uzma *et al.* 2025). Critically, both deficient and excessive IAA levels can impair plant development by disrupting auxin homeostasis. Low IAA concentrations can reduce primary root elongation and suppress lateral root formation, thereby limiting nutrient uptake (Fu *et al.* 2015; Liu *et al.* 2023; Yang *et al.* 2025). Excessively high IAA, however, may inhibit primary root growth, disrupt cellular elongation, and induce hormonal stress responses (Roychoudhry and Kepinski 2021; Ashokkumar *et al.* 2025). Therefore, maintaining a moderate auxin concentration is essential for balanced root development and overall plant performance (Pantoja-Guerra *et al*. 2023). In this context, the moderate but consistent IAA production by KBK3 represents a functionally meaningful trait. It is sufficient to stimulate positive morphological changes without triggering inhibitory stress responses, underscoring the strain's suitability for development as a balanced and effective bioinoculant for sustainable agriculture.

The physiological profile of KBK3 indicates a broad adaptive capacity. Notably, KBK3's ability to grow

at 10.5% NaCl surpasses the tolerance of other environmental *Aeromonas* strains, such as *Aeromonas* sp. FONT1B and FONT3B, which fail to grow at 5% NaCl, though *Aeromonas* sp. FONT6 can tolerate up to 10% (Antonelli *et al.* 2025). Its growth across a wide pH range (4-9) is consistent with other resilient strains like FONT6 and strains A1-2 and C7_8, which also grow at pH 4 and 10 (Agunbiade *et al*. 2024; Antonelli *et al.* 2025). The pathogenic *Aeromonas veronii* shows a similar pH range (5-9) but markedly weaker halotolerance, with poor growth above 3% NaCl (Hassan *et al.* 2017). While some *Aeromonas* strains exhibit survival at extremely low temperature (-72°C) (Abeyta *et al.* 1986), KBK3's optimal growth at 35°C aligns with other PGPR *Aeromonas* strains (Agunbiade *et al.* 2024). Importantly, its sustained growth under high osmotic stress (30% PEG) mirrors the notable drought tolerance reported for strains A1-2 and C7_8 (Agunbiade *et al.* 2024). Collectively, these results position *Aeromonas* sp. KBK3 as a robust PGPR candidate with notable abiotic stress resilience, enhancing its potential for application as a bioinoculant in suboptimal or variable soil conditions.

The isolate *Aeromonas* sp. KBK3 further demonstrated a capacity to enhance plant nutrition through phosphate solubilization. Phosphorus (P) is a critical macronutrient for fundamental processes such as photosynthesis and cell division; however, its bioavailability in soil is severely limited, with only 0.1-5% existing in the soluble orthophosphate forms ($H_2PO_4^-$ and HPO_4^{2-}) accessible to plants (Hakim *et al.* 2021; Alzate Zuluaga *et al*. 2024). KBK3 likely mobilizes insoluble soil phosphates via a common PGPR mechanism: the secretion of low-molecular-weight organic acids (e.g., gluconic or citric acid). These compounds acidify the microenvironment and chelate metal cation (e.g., Ca^{2+}, Fe^{3+}, Al^{3+}), thereby releasing plant-available phosphate(Chandran *et al*. 2021; Saeed *et al.* 2021). This function aligns with well-characterized phosphate-solubilizing genera, including *Pseudomonas*, *Bacillus*, and *Streptomyces* (Mahdi *et al.* 2020; Soumare *et al.* 2021; Timofeeva *et al.* 2023; Wahab *et al.* 2024). The absence of direct antagonistic traits like HCN and chitinase production in KBK3 suggests its primary ecological role is nutrient facilitation rather than pathogen inhibition. Together, these multifunctional traits solidify KBK3's profile as a promising bioinoculant for improving plant nutrition and growth under nutrient-limiting conditions.

The enzymatic profile of *Aeromonas* sp. KBK3 further clarifies its functional niche within the rhizosphere. The production of cellulase and pectinase indicates a capacity to degrade key plant-derived polymers, contributing to organic matter turnover and nutrient cycling. Cellulase activity facilitates the breakdown of cellulose into simpler sugars, supporting soil carbon dynamics (Hakim *et al*. 2021; Shi *et al*. 2022; De Andrade *et al*. 2023; Sritongon *et al*. 2023). Pectinase, in turn, aids in the decomposition of pectin, a major component of plant cell walls, thereby promoting carbon mineralization and the release of nutrients from plant residue (El Hjouji *et al*. 2025; Hajji-Hedfi *et al*. 2025). Notably, pectinolytic activity has been positively correlated with increased rhizosphere colonization through biofilm formation (Shi *et al*. 2022). Conversely, the absence of detectable protease, chitinase, and amylase activities suggests a limited capacity for direct enzymatic antagonism against pathogens. Protease and chitinase are particularly associated with the biocontrol of fungal pathogens, as they degrade essential structural proteins and chitin in cell walls (Jadhav *et al*. 2017; Chandran *et al.* 2021; Hamada and Soliman 2023). The lack of these enzyme positions KBK3 closer to PGPR strains primarily involved in nutrient facilitation and organic matter transformation, rather than these specializing in direct pathogen suppression (Hakim *et al*. 2021; Hajji-Hedfi *et al*. 2025).

The functional profile of *Aeromonas* sp. KBK3, characterized by the absence of nitrogen fixation and hydrogen cyanide (HCN) production, further refines its ecological specialization toward mineral solubilization and organic matter decomposition rather than atmospheric nitrogen assimilation or direct cyanogenic antagonism. This does not detract from its plant growth-promoting potential, as many effective PGPR strains facilitate growth through mechanisms independent of biological nitrogen fixation. In non-leguminous systems, nitrogen acquisition often occurs via alternative pathways, and various beneficial bacteria, including some rhizobia, can promote growth in non-host plants through diverse direct and indirect mechanisms (Solomon *et al*. 2024). Similarly, the lack of detectable HCN aligns with finding for other highly effective PGPR isolate where cyanogenesis is not a prerequisite for plant-beneficial activity (Agunbiade *et al*. 2024). This absence may be advantageous, as it reduces potential risk while underscoring that KBK3's primary mode of action is likely rooted in enhancing nutrient bioavailability and participating in rhizosphere

nutrient cycling, rather than in pathogen suppression via cyanide production.

Consistent with its nutrient-facilitating profile, *Aeromonas* sp. KBK3 exhibited no adverse effects on seed germination relative to the control, confirming its non-phytotoxic nature and compatibility with early seedling development. This non phytotoxic germination response aligns with reports for other mineral-solubilizing PGPR, such as strains of *Klebsiella, Enterobacter, Pseudomonas, Bacillus*, and *Serratia* (Mahdi *et al*. 2020; Fahsi *et al*. 2021; Salazar-Ramírez *et al*. 2021; Fiodor *et al*. 2023; Ganesh *et al*. 2024; Bhardwaj *et al*. 2025), further supporting its safety and potential for use as an agricultural inoculant.

5. CONCLUSION

This study identifies and characterizes *Aeromonas* sp. KBK3, a novel and robust plant growth-promoting rhizobacterium (PGPR) isolated from the banana rhizosphere. The isolate demonstrates a multifaceted profile of agronomically valuable traits. Its high potassium solubilization capacity (KSI 3.77 ± 0.27 within 4 days), combined with moderate indole-3-acetic acid production, and phosphate solubilization, directly addresses key constraints in plant nutrient acquisition. Furthermore, its enzymatic activities (cellulase, pectinase) suggest a complementary role in rhizosphere carbon cycling. The strain exhibits pronounced resilience to abiotic stressors, including salinity, pH variation, and osmotic drought—highlighting its adaptability to challenging soil environments. Critically, its non-phytotoxic nature, confirmed by a neutral effect on seed germination, ensures biological safety for agricultural application.

Collectively, the functional attributes, stress tolerance, and biosafety of *Aeromonas* sp. KBK3 underscore its significant potential as a next-generation microbial inoculant. It is a promising candidate for development into a biofertilizer aimed at enhancing nutrient use efficiency and crop resilience. Subsequent validation through controlled greenhouse trials and field studies is essential to confirm its efficacy and integrate it into sustainable crop production systems.

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