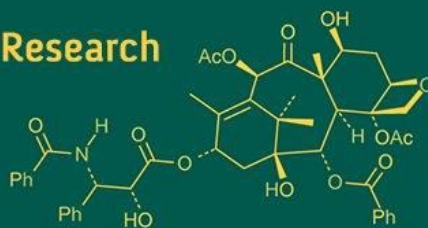


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Isolation, Morphocultural and biochemical characterization of N-fixing, P-solubilizing and K-mobilizing bacteria from brinjal rhizosphere

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Abstract

This manuscript details the isolation and characterization of nitrogen-fixing, phosphate-solubilizing, and potassium-mobilizing bacteria from brinjal rhizosphere soils across Nashik, Pune, and Ahmednagar districts in Maharashtra, India. Soil samples underwent serial dilution and selective pour plating on Jensen's, Pikovskaya's, and Aleksandrov's media respectively, yielding 20 N-fixers, 15 PSB (primarily *Bacillus/Pseudomonas* spp.) isolates, and 13 K-mobilizers, which were further purified, maintained and assessed for morphological traits and biochemical profiling. These diverse PGPR strains demonstrate strong nutrient-solubilizing potential via halo zones and quantitative assays, positioning promising isolates like AZO16, PSB4, and KMB5 for biofertilizer consortia to enhance brinjal nutrition and promote sustainable farming over chemical inputs.

Keywords: Biofertilizer, Azotobacter, Bacillus, PGPR

1. Introduction

Brinjal (*Solanum melongena* L.) is a native to India, Central Asia and cultivated extensively in India, Indonesia, Philippines and some African countries. Apart from its culinary versatility brinjal is also known for its nutritional and functional properties. It is of low caloric density with high carbohydrates, dietary fiber, proteins, vitamin A, vitamin B and vital minerals like potassium, calcium and iron (Kaur *et al.*, 2024) ^[1]. Brinjal is cultivated over 685 thousand hectares with a production of 13074 thousand MT (3rd advance estimate of Ministry of Agriculture and Farmers' Welfare, Government of India, 2024-2025). Despite of production, presently brinjal production faces several challenges like inadequate nutrient supply, biotic and abiotic stresses that ultimately impacts yield potential and product quality. In consideration of expanding worldwide population and climate change scenario, retaining the highest yield with quality of vegetables remains a foremost concern for sustainable agriculture. Bio-fertilizers are renowned as a justifiable alternative to chemical fertilizers which are capable of enhancing plant growth by improving soil fertility and plant nutrition through multiple mechanisms simultaneously. They include atmospheric nitrogen fixation, inorganic phosphate solubilization, potassium mobilization that increases nutrient availability and sustain soil health. Most of the microorganisms also produces various of plant growth regulators viz., indole-3-acetic acid (IAA) and gibberellins (Kaur *et al.*, 2024) ^[1] as well as synthesizes siderophores for micronutrient bioavailability, mitigate abiotic and biotic stressess and induced systemic resistance contrary to pathogens.

Various non symbiotic nitrogen fixing diazotrophic bacterial genera *Azotobacter*, *Burkholderia*, *Azoarcus*, *Azospirillum*, *Gluconacetobacter*, *Diazotrophicus*, *Pseudomonas* and *Enterobacter*, as well as cyanobacteria such as *Anabaena* fixes atmospheric nitrogen in non-legumes (Shahwar *et al.*, 2023) ^[2]. Soil available pool of soil phosphorus in the form of inorganic orthophosphate ions (monobasic H₂PO₄ or dibasic HPO₄²⁻) is principally solubilized by *Bacillus* spp. such as *B. subtilis*, *B. cereus*, *B. polymyxa*, *B. circulans*, *B. circulmou* and *B. megaterium* (Kucey *et al.*, 1989 and Mohamed *et al.* 2018) ^[3, 4]. Potassium-mobilizing bacteria (KMB) include different group of microorganisms including *Frateruria aurantia*., *Bacillus subtilis*, *B. mucilaginosus*, *B. cereus*, and, *B. pumilus*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*, which solubilize mineral-bound potassium by chelation mechanisms and

organic acid secretion (Jha, 2017; Deshmukh *et al.*, 2020)^[5, 6]. The present study includes isolation and characterization strategies of beneficial PGPR strains isolated from native rhizospheric soils among brinjal growing pockets of Maharashtra.

2. Materials and Methods

2.1 Experimental site

Present study was conducted in the Department of Plant Pathology and Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri (MS) India.

2.2 Collection of rhizospheric soil samples from onion rhizosphere

Rhizospheric soil samples were collected from major brinjal growing villages in Nashik, Pune and Ahilyanagar districts of Maharashtra to isolate nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria. At a depth of 10 to 15 cm below the surface and soil samples were collected in sampling bags. Further, these soil samples were air dried and crushed in wooden mortar and pestle. Soil was sieved through a 4 mm mesh sieve and stored at 4 °C.

2.3 Isolation of N fixing, P solubilizing and K mobilizing bacteria from rhizosphere soil samples

Isolation was carried out by using a method described by Aneja (2003)^[7]. 10 g air-dried rhizosphere soil samples were suspended in 90 mL sterile distilled water, agitated vigorously, and serially diluted from 10^{-1} to 10^{-6} . A 1 mL aliquots from dilutions 10^{-3} to 10^{-6} were plated on sterilized petriplates. A sterilized medium before solidification at 45 °C, was poured into each petriplate. pour-plated onto selective media: Jensen's nitrogen-free agar for N-fixers, Pikovskaya's agar for P-solubilizers, and Aleksandrov's agar for K-solubilizers. Media were sterilized, cooled to 45 °C, poured over suspensions, swirled gently without lid contact, solidified, and incubated at 28 ± 2 °C for 3-5 days. Colonies showing growth on nitrogen-free medium or clear halos around solubilization zones were selected, purified by quadrant streaking, and maintained as slant cultures for downstream characterization. This standardized serial dilution and pour-plate approach, adapted from established protocols, ensures efficient recovery of plant growth-promoting rhizobacteria from brinjal rhizosphere. Identification proceeded via morpho-biochemical assays as per Pal *et al.*, (2017)^[8].

2.4 Purification, multiplication and maintenance of bacterial culture

A loopful bacterial growth of nitrogen fixing, phosphate and potassium solubilizing bacteria was streaked on Jensen's, Pikovskaya's and Alexandrov's media separately and incubated at 28 °C for colony development. Further, Isolates were subsequently transferred to new plates to examine growth and to check inhibition zones (if any). These purified bacterial cultures were preserved for further studies.

2.5 Characterization of pure isolates

2.5.1 Cultural and Morphological characterization of isolates

Bacterial microorganisms were recognized on the cultural, morphological and biochemical characters by following Bergey's Manual (2nd ed., 2009) by De Vos and Garrity (2009)^[9]. The nitrogen fixing, phosphate solubilizing and potassium mobilizing isolates were studied for their cell shape, size, arrangement and motility. Overnight cultures

were streaked on Jensen's, Pikovskaya's and Alexandrov's media and incubated at 28 ± 2 °C for 48 hours to observe colony morphology which includes growth pattern, form, elevation and margin.

2.5.2 Biochemical characterization of isolates

The bacterial isolates were subsequently characterized biochemically as per the techniques in the 10th edition of Microbiology: A Laboratory Manual by Cappuccino and Sherman (1987)^[10]. Several tests comprising the Gram staining, Catalase test, Oxidase test, Indole production test, Methyl Red test, Voges-Proskauer (VP) test, Urease test, Nitrate reduction test, Siderophore production and H₂S production test was performed, by following methodologies by Rosemary *et al.* (2013)^[11], Upadhyay *et al.* (2015)^[12] and Muthukumar *et al.* (2021)^[13].

2.5.3 Gram's staining

The categorization of these isolates were performed using the Gram staining technique stated by Rosemary *et al.* (2013)^[11] and Parunandi *et al.* (2023)^[14]. A loopful of the culture was taken onto a clean, dry slide and a smear was prepared, air-dried and then heat-fixed. As main stain, two drops of crystal violet were added and left for 30 seconds, subsequently distilled water washed. Afterward, 1-2 drops of Gram's iodine were added and left the slide for 60 seconds, followed by 95% ethyl alcohol washed. A counterstain safranin was then added and left the preparation for 30 seconds and washed with distilled water. The slide was dried by using blotting paper and observed under a microscope. Purple colonies were indicated as Gram-positive bacteria while pink coloured colonies specified Gram-negative bacteria.

2.5.4 Catalase test

Cultured organisms sample was moved to a labelled slide, subsequently the addition of hydrogen peroxide 3% drops. The instantly formed bubble indicates a positive catalase test.

2.5.5 Oxidase test

Three drops of p-amino dimethylaniline oxalate were placed on the growth surface of a microorganism. The area was detected for a color change within 20-30 seconds. Appearance of a purple colour within 10-30 seconds the test was found a positive result, while the absence of color change indicated a negative result.

2.5.6 Indole production test

The cultured microorganism was stab-inoculated in to Tryptone broth in a deep tube. These tubes were further incubated at 37 °C for 24 to 48 hours. After incubation, 10 drops of Kovac's reagent were applied to each tube. The appeared red layer at the surface showed a positive indole test, in negative test reaction no any red layer formed.

2.5.7 Methyl red test

The growth of cultured microorganism was transferred to a labelled tube comprising MR-VP broth and incubated at 37 °C for 24 to 48 hours. Next to incubation, inoculation tube was added with five drops of methyl red indicator. Appearance of a red colour, indicating a pH around 4, signified a positive result. Contrary, a yellow colour, indicating a pH of 6, was denotes a negative result, as it still advocated presence of acid but with a reduced hydrogen ion concentration.

2.5.8 Voges-Proskauer test

Test cultured microorganisms were inoculated with a MR-VP broth tube and further incubated at 37 °C for 24 to 48 hours. Afterwards, 10 drops each of Barritt's reagent A and Barritt's reagent B were added consecutively. The positive result indicated by development of a deep rose color within 15 minutes for acetoin (acetylmethylcarbinol) production, while the absence of color change indicated a negative result.

2.5.9 Urease test

The cultured were inoculated overnight with sterilized urea broth tubes and subsequently incubated at 50 ± 2 °C for 48 hours. A change in colour to pink specified a positive urease test, while no change in color was documented as negative. A pre-sterilized, uninoculated urea broth considered as the control.

2.5.10 Nitrate reduction test

Cultured test microorganism was stab-inoculated into a deep tube of SIM agar followed by incubation at 37 °C for 24 to 48 hours. After incubation, 10 drops of Kovac's reagent were applied. A red layer at the surface indicated a positive indole test, while the absence of a red layer indicated a negative reaction.

2.5.11 H₂S test

The test microorganism was stab-inoculated into a SIM agar medium tube and incubated at 37 °C for 24 to 48 hours. For this H₂S production test, a medium containing ferrous ammonium sulphate served as an indicator which formed an insoluble black ferrous sulphide precipitate along with the stab line in the presence of hydrogen sulphide gas. While absence of black precipitate specified a negative reaction.

2.5.12 Siderophore production

Siderophore production was estimated qualitatively (Yeole and Dube, 2000) [15]. A 0.5 percent of cell free culture supernatant was added to 0.5 mL of 0.2 percent aqueous Ferric chloride solution. Appearance of orange or reddish brown colour indicated the presence of siderophore, considered as positive for siderophore.

2.6 Identification of efficient strains of nitrogen fixer, phosphate solubilizer and potash mobilizer

For N fixing bacteria, a 48-h-old culture of freshly isolated, grown on nitrogen-free Jensen's media was subjected to total nitrogen estimation using the standard Micro-Kjeldahl procedure. For PSB, the bacterial isolates were cultured on Pikovskaya's selective agar medium for 4 days at 28 °C and assessed for phosphate solubilizing activity and amount of Pi liberated from TCP in broth medium was quantified by following (Jackson, 1973) [16]. The appearance of a halo zone around the colony indicated phosphate solubilization. In case of KMB by following zone of potash solubilisation on alexandrov agar medium further the amount of K released from muscovite mica by the isolates was assessed at 7, 15 and 20 days after incubation (DAI) under laboratory conditions, following the method described by Parmar (2016) [17].

3. Results and Discussion

In the current investigation, 36 rhizosphere soil samples were collected from diverse villages spanning three districts Nashik, Pune and Ahilyanagar of Maharashtra guided by precise GPS coordinates. These specimens facilitated the

isolation and characterization of key beneficial microbes, including nitrogen-fixing, phosphate-solubilizing, and potash-mobilizing bacteria.

3.1 Isolation of N fixing, P solubilizing and K mobilizing bacteria

Nitrogen-fixing bacteria were isolated from brinjal rhizosphere soil samples employing Jensen's nitrogen-free agar medium. Distinct milky-white colonies that emerged were sub cultured onto freshly prepared Jensen's plates and incubated at 28 ± 2 °C for 48-72 h, which yielded a total of 20 distinct isolates of N fixing bacteria. Similar outcomes align with Jain *et al.* (2021) [18], who recovered 172 *Azotobacter* strains from varied rhizospheric soils using serial dilution on Jensen's medium. These findings corroborate reports by Priya *et al.* (2013) [19], Sharma *et al.* (2018) [20], Samal *et al.* (2020) [21], and Lolita *et al.* (2022) [22], which employed comparable serial dilution techniques on nitrogen-deficient media (Jensen's or Burk's agar) and incubation at 28 ± 2 °C for 3-7 days to isolate diazotrophs from brinjal rhizospheres.

Phosphate-solubilizing bacteria (PSB) were isolated using Pikovskaya's agar medium amended with tricalcium phosphate (TCP). Among 36 brinjal rhizosphere samples, yielding 15 efficient PSB isolates based on halo formation. Analogous findings on isolation and characterization of PSB were also led by Amri *et al.* (2023) [23] and Mengesha and Legesse (2024) [24]. A total of thirteen bacterial isolates (KMB1 to KMB13) demonstrating efficient potassium mobilizing ability were retrieved by using Alexandrov's medium containing muscovite mica as insoluble potassium source through serial dilution and pour plate technique following Hu *et al.* (2006) [25]. Table 1 indicates the details of sample isolated from soil.

3.2 Cultural, morphological and biochemical characterization of nitrogen fixing bacterial isolates

All isolates established growth on nitrogen-free medium, confirming their nitrogen-fixing capability. Table 2. represents cultural and morphological characteristics of isolates to detect phenotypic variations. The isolates shown varied colony morphology in terms of shape, colour, texture, margin, elevation and size. Diverse colony pigmentation from white and creamy to yellow and creamy white appears, while circular to irregular forms observed with raised or convex colonies followed by entire or undulate margins which aligns with Jain *et al.* (2021) [18] and Upadhyay *et al.* (2015) [12].

All 20 nitrogen-assimilating strains (AZO1-AZO20) displayed uniform Gram-negative bacilli morphology (Table 3), alongside ubiquitous catalase reactivity (100%) a mark of oxidative stress tolerance in *Azotobacter*-like taxa and oxidase proficiency (100%), enabling aerobic respiration in soil microsites. Indole reactivity varied (45% positive), reflecting differential tryptophan catabolism, while N₂ reduction prevailed in 85% of strains, affirming diazotrophy; urease activity reached 90%, aiding N cycling via urea hydrolysis.

H₂S production showed balance (55% positive), indicative of sulphur versatility, with Voges-Proskauer acetoin response in 75% and methyl red acid fermentation in 80%, denoting mixed glucose metabolism. Siderophore secretion occurred in 75%, crucial for Fe scavenging and root colonization synergy. Profiles align with Jain *et al.* (2021) [18], who noted Gram-ve, catalase + /oxidase + *Azotobacter* from arid soils, and Jiménez *et al.* (2011) [26] reporting variable indole/H₂S in organic farm diazotrophs

corroborating presumptive *Azotobacter chroococcum* dominance (e.g., AZO16: fully reactive except H₂S/VP/MR) for biofertilizer consortia. This diversity underpins strain selection for enhanced N delivery in brinjal systems.

3.3 Cultural, morphological and biochemical characterization of phosphate solubilizing bacterial isolates

Table 4. contains a total of fifteen bacterial isolates (PSB1 to PSB15) found rod shaped and shows motility with white-creamy white to yellow colony pigmentation and circular colonies with variable margins. This finding is align with observations of Amri *et al.* (2023) [23].

In Table 5, the 15 phosphate-mobilizing isolates (PSB1-PSB15) exhibited Gram reaction variability (47% positive, 53% negative), typifying *Bacillus-Pseudomonas* assemblages, with complete catalase (100%) and oxidase (100%) reactivity key for oxidative P mineralisation in aerated soils. Indole formation occurred in 47%, signalling diverse aromatic metabolism, while nitrate reduction prevailed (60%) and urease hydrolysis reached 87%, bolstering rhizosphere N availability.

Test for H₂S production balanced at 53%, reflecting sulphur plasticity, alongside near-universal acetoin generation (87% VP-positive) and mixed-acid fermentation (80% methyl red-reactive), hallmarks of *Bacillus* fermentative vigour. Siderophore elaboration marked 80% of strains, vital for Fe-P synergism in nutrient-scarce matrices. These traits parallel Saini *et al.* (2025) [27] on catalase/oxidase-dominant PSB and Amri *et al.* (2023) [23] noting Gram-dual, siderophore-

prolific rice rhizobacteria, positioning strains enhancing P uptake.

3.4 Cultural, morphological and biochemical characterization of potash solubilizing bacterial isolates

All thirteen isolates were shows rod-shaped morphology and predominantly exhibited white or yellow pigmentation which align with the findings by Parunandi *et al.* (2023) [14]. The circular to irregular with entire (smooth) margins colony form was observed with mostly raised elevation, although a few demonstrated a flat profile in table 6.

Among 13 potassium-mobilizing isolates (KMB1-KMB13), Gram status varied (77% positive, 23% negative), aligning with *Bacillus-Frateuria* spectra, coupled with invariant catalase reactivity (100%) for H₂O₂ scavenging during mica weathering. Oxidase response was modest (46%), suiting microaerophilic K release, while indole positivity hit 85% promoting auxin-like signalling and nitrate denitrification 62%, with urease at 46% for alkaline solubilization aid.

H₂S output remained low (15%), emphasizing acid-driven K chelation over sulfidogenesis, with robust acetoin (100% VP) and acid production (92% methyl red), fostering fermentative mineral attack. Siderophore yield reached 100%, amplifying multi-nutrient mobilization. Patterns resonate with Parunandi *et al.* (2023) [14] on Gram-ve/ + KSB with catalase ubiquity and Thuvaraka *et al.* (2023) [28] highlighting VP-dominant mica degraders, designating versatile strains for consortium integration boosting brinjal K nutrition.

Table 1: Details of soil sampling sites for isolation of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria from brinjal rhizosphere

Coding used for soil samples	District	Tehsil	Village	Latitude	Longitude
NSP1	Nashik	Sinnar	Pandhurli	19.8298° N	73.8544° E
NSM2	Nashik	Sinnar	Mhalungi	19.77° N	73.96° E
NNB3	Nashik	Nashik	Belgaon Dhaga	19.9677° N	73.6888° E
NDV4	Nashik	Dindori	Varkheda	20.2349° N	73.8933° E
NDK5	Nashik	Dindori	Khadaksukene	20.21° N	73.86° E
NDM6	Nashik	Dindori	Mohadi	20.1472° N	73.8882° E
NDJ7	Nashik	Dindori	Janori	20.1053° N	73.8871° E
NSJ8	Nashik	Satana	Jaikheda	20.5905° N	74.1987° E
NNPB9	Nashik	Niphad	Pimpalgaon baswant	20.1667° N	73.9833° E
NNV10	Nashik	Niphad	Vinchur	20.1059° N	74.2355° E
NYS11	Nashik	Yeola	Adgaon	20.0392° N	73.8566° E
NYS12	Nashik	Yeola	Savargaon	20.1000° N	74.4500° E
PJM13	Pune	Junnar	Manglur Pargaon	19.0425° N	74.1544° E
PJO14	Pune	Junnar	Otur	19.2606° N	73.9940° E
PJJ15	Pune	Junnar	Junnar	19.2082° N	73.8742° E
PAK16	Pune	Ambegaon	Kalamb	18.5745° N	76.0434° E
PAM17	Pune	Ambegaon	Manchar	19.0044° N	73.9435° E
PAM18	Pune	Ambegaon	Mahalunge Padawal	18.5745° N	76.0434° E
PRC19	Pune	Rajgurunagar	Chas Kaman	18.8666° N	73.9000° E
PRK20	Pune	Rajgurunagar	Kharpudi Bk.	18.8182° N	73.9183° E
PBS21	Pune	Baramati	Supe	18.3346° N	74.3775° E
PBP22	Pune	Baramati	Palshi	18.2344° N	74.4520° E
PSS23	Pune	Shirur	Mandavgan Pharata	18.5549° N	74.4898° E
PDP24	Pune	Daund	Patas	18.4334° N	74.4649° E
ARS25	Ahilyanagar	Rahuri	Satral	19.5151° N	74.4894° E
ARK26	Ahilyanagar	Rahuri	Kolhar	19.5146° N	74.5316° E
ARD27	Ahilyanagar	Rahata	Dadh Bk	19.5269° N	74.4119° E
ARSA28	Ahilyanagar	Rahata	Sakori	19.7325° N	74.4775° E
ASJ29	Ahilyanagar	Sangamner	Jorve	19.5811° N	74.2050° E
ASD30	Ahilyanagar	Sangamner	Dhandarphal	19.5460° N	74.1215° E
ASB31	Ahilyanagar	Shrirampur	Belapur	19.5886° N	74.6371° E
ASN32	Ahilyanagar	Shrirampur	Nimgon Khairi	19.6808° N	74.6553° E
ANN33	Ahilyanagar	Nevasa	Najik Chincholi	19.5333° N	74.9333° E
ANP34	Ahilyanagar	Nevasa	Pichadgaon	19.5243° N	75.0077° E
APN35	Ahilyanagar	Parner	Nighoj	18.9270° N	74.2905° E
ASP36	Ahilyanagar	Shrigonda	Pargaon	18.6160° N	74.6981° E

Table 2: Colony characterization of nitrogen fixing bacterial isolates

Sr. No.	Name of the isolate	Colony characteristics					
		Morphological			Cultural		
		Shape	Colour	Motility	Form	Margin	Elevation
1	AZO1	Rod	whitish	Motile	Circular	Entire	Convex
2	AZO2	Rod	Creamy	Motile	Circular, raised	Entire	Convex
3	AZO3	Rod	Dull white	Motile	Circular	Entire	Convex
4	AZO4	Rod	whitish	Motile	Circular	Entire	Convex
5	AZO5	Rod	whitish	Motile	Irregular	Entire	Raised
6	AZO6	Rod	whitish	Motile	Irregular	Entire	Raised
7	AZO7	Rod	creamy	Motile	Circular	Undulate	Convex
8	AZO8	Rod	whitish	Motile	Circular	Entire	Convex
9	AZO9	Rod	whitish	Motile	Circular	Entire	Raised
10	AZO10	Rod	Dull white	Motile	Circular	Undulate	Convex
11	AZO11	Rod	whitish	Motile	Circular	Entire	Convex
12	AZO12	Rod	whitish	Motile	Circular	Entire	Convex
13	AZO13	Rod	whitish	Motile	Circular	Entire	Raised
14	AZO14	Rod	Yellowish white	Motile	Circular	Undulate	Convex
15	AZO15	Rod	Dull white	Motile	Circular	Undulate	Convex
16	AZO16	Rod	whitish	Motile	Irregular	Entire	Convex
17	AZO17	Rod	whitish	Motile	Circular	Entire	Convex
18	AZO18	Rod	whitish	Motile	Circular	Entire	Convex
19	AZO19	Rod	Creamy white	Motile	Circular	Entire	Convex
20	AZO20	Rod	whitish	Motile	Circular	Entire	Convex

Table 3: Biochemical characterization of Nitrogen Fixing Bacterial isolates

Sr. No.	Isolate	Grams reaction	Catalase	Oxidase	Indole	N ₂ Reduction	Urease	H ₂ S	VP test	Methyl red	Siderophore Production
1	AZO1	-ve	+	+	-	+	+	+	-	+	+
2	AZO2	-ve	+	+	-	+	+	+	+	+	+
3	AZO3	-ve	+	+	+	+	+	+	+	+	+
4	AZO4	-ve	+	+	+	-	-	-	+	+	+
5	AZO5	-ve	+	+	+	+	+	+	+	+	+
6	AZO6	-ve	+	+	-	-	+	-	-	-	-
7	AZO7	-ve	+	+	-	+	+	+	+	-	-
8	AZO8	-ve	+	+	-	+	-	-	-	+	+
9	AZO9	-ve	+	+	+	-	+	+	+	+	+
10	AZO10	-ve	+	+	-	-	+	-	-	-	-
11	AZO11	-ve	+	+	-	+	+	+	+	+	-
12	AZO12	-ve	+	+	+	+	+	+	+	+	+
13	AZO13	-ve	+	+	-	+	+	-	+	+	+
14	AZO14	-ve	+	+	-	-	+	+	+	+	+
15	AZO15	-ve	+	+	+	+	+	+	+	+	+
16	AZO16	-ve	+	+	+	+	+	-	-	-	+
17	AZO17	-ve	+	+	+	+	+	-	+	-	-
18	AZO18	-ve	+	+	-	+	+	-	+	+	+
19	AZO19	-ve	+	+	-	-	+	+	+	+	+
20	AZO20	-ve	+	+	+	+	+	+	+	+	+

Note: + positive test,-negative test**Table 4:** Colony characterization of phosphate solubilizing bacterial isolates

Sr. No.	Name of the isolate	Colony characteristics					
		Morphological			Cultural		
		Shape	Colour	Motility	Form	Margin	Elevation
1	PSB1	Rod	White	Motile	Circular	Entire	Raised
2	PSB2	Rod	White	Motile	Circular	Lobate	Flat
3	PSB3	Rod	White	Motile	Circular	Entire	Raised
4	PSB4	Short Rod	Creamy white	Motile	Circular	Entire, smooth	Raised
5	PSB5	Rod	Yellow	Motile	Circular	Lobate	Raised
6	PSB6	Rod	White	Motile	Circular	Entire	Raised
7	PSB7	Rod	White	Motile	Circular	Lobate	Convex

8	PSB8	Rod	Yellow	Motile	Circular	Lobate	Convex
9	PSB9	Rod	Yellow	Motile	Circular	Lobate	Flat
10	PSB10	Rod	White	Motile	Circular	Lobate	Flat
11	PSB11	Rod	White	Motile	Circular	Entire	Flat
12	PSB12	Rod	White	Motile	Circular	Entire	Raised
13	PSB13	Rod	Yellow	Motile	Circular	Lobate	Flat
14	PSB14	Rod	Yellow	Motile	Circular	Lobate	Flat
15	PSB15	Rod	White	Motile	Circular	Lobate	Flat

Table 5: Biochemical characterization of phosphate solubilizing bacterial isolates

Sr. No.	Isolate	Grams reaction	Catalase	Oxidase	Indole	N ₂ Reduction	Urease	H ₂ S	VP test	Methyl red	Siderophore Production
1	PSB1	+Ve	+	+	+	+	+	+	+	+	+
2	PSB2	+Ve	+	+	+	-	+	+	+	+	+
3	PSB3	-Ve	+	+	+	-	+	+	+	+	-
4	PSB4	+Ve	+	+	-	+	-	-	+	-	+
5	PSB5	-Ve	+	+	+	+	+	+	+	+	+
6	PSB6	+Ve	+	+	-	-	+	+	+	+	+
7	PSB7	+Ve	+	+	+	+	+	+	+	+	-
8	PSB8	-Ve	+	+	+	+	+	-	+	+	+
9	PSB9	+Ve	+	+	+	+	+	+	+	+	+
10	PSB10	+Ve	+	+	+	+	+	+	+	+	+
11	PSB11	-Ve	+	+	-	-	+	+	-	+	+
12	PSB12	-Ve	+	+	-	-	+	-	+	+	+
13	PSB13	-Ve	+	+	-	-	+	+	+	+	-
14	PSB14	+Ve	+	+	-	+	+	+	+	-	-
15	PSB15	+Ve	+	+	-	+	+	+	+	-	+

Note: + positive test, - negative test**Table 6:** Colony characterization of Potash mobilizing bacterial isolates

Sr. No.	Name of the isolate	Colony characteristics					
		Morphological			Cultural		
		Shape	Colour	Motility	Form	Margin	Elevation
1	KMB1	Rod	Yellow	Motile	Circular	Entire	Raised
2	KMB2	Rod	White	Motile	Irregular	Entire	Raised
3	KMB3	Rod	White	Motile	Circular	Entire	Raised
4	KMB4	Rod	White	Motile	Circular	Entire	Raised
5	KMB5	Rod	Creamy White	Motile	Circular	Entire	Flat
6	KMB6	Rod	White	Motile	Irregular	Entire	Raised
7	KMB7	Rod	White	Motile	Irregular	Entire	Raised
8	KMB8	Rod	Yellow	Motile	Circular	Entire	Raised
9	KMB9	Rod	White	Motile	Circular	Entire	Flat
10	KMB10	Rod	White	Motile	Irregular	Entire	Raised
11	KMB11	Rod	White	Motile	Circular	Entire	Raised
12	KMB12	Rod	Yellow	Motile	Circular	Entire	Raised
13	KMB13	Rod	White	Motile	Circular	Entire	Flat

Table 7: Biochemical characterization of potash mobilizing bacterial isolates

Sr. No.	Isolate	Grams reaction	Catalase	Oxidase	Indole	N ₂ Reduction	Urease	H ₂ S	VP test	Methyl red	Siderophore Production
1	KMB1	-ve	+	-	+	+	-	-	+	+	+
2	KMB2	+ve	+	+	+	+	-	+	+	+	+
3	KMB3	+ve	+	+	+	+	+	+	+	+	+
4	KMB4	+ve	+	-	+	+	-	-	+	+	+
5	KMB5	+ve	+	-	-	-	-	-	+	-	+
6	KMB6	+ve	+	-	+	+	-	-	+	+	+
7	KMB7	+ve	+	-	+	+	+	-	+	+	+
8	KMB8	-ve	+	-	+	-	-	-	+	+	+
9	KMB9	+ve	+	+	+	-	+	+	+	+	+
10	KMB10	+ve	+	+	+	+	-	+	+	+	+
11	KMB11	+ve	+	-	+	+	-	-	+	+	+
12	KMB12	-ve	+	+	+	-	+	+	+	+	+
13	KMB13	+ve	+	-	-	-	-	-	+	-	+

Note: + positive test, - negative test.**Conclusion**

In the present study, a varied rhizobacteria viz., nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria were isolated from brinjal rhizospheric soils were

collected across three brinjal growing districts pockets in Maharashtra. By using selective media and performing standard microbiological techniques, pure bacterial colonies were obtained, further purified and maintained for further

study. Cultural, morphological and biochemical characterizations of isolates provide extensive morphological diversity among the isolates. Mostly, nitrogen fixing bacterial isolates were characterized as Gram-negative rods (mostly *Azotobacter* spp.), While phosphate solubilizing and potash mobilizing bacteria's were rods shaped, having motility with variable Gram reactions (mostly *Bacillus* or *Pseudomonas*) and included both rods and cocci. The experimental variability in colony morphology, pigmentation, motility and biochemical characters indicates the presence of multiple efficient rhizobacterial strains with nutrient solubilizing potential and ultimately agronomic significance. On the basis of quantitative evaluation for nitrogen fixation capacity, phosphate solubilization efficacy using Pikovskaya's agar medium and potassium solubilization efficacy using modified Aleksandrov's agar medium isolate AZO16, PSB4 and KMB5 emerge as potential isolates which were further used to design bacterial consortium upon their compatibility study. These outcomes supported by earlier reports and emphasize the importance of non-legume rhizobacteria in sustainable agriculture. The evaluated strains demonstrated their potential to emerge as biofertilizer formulations intended in improving soil fertility and ultimately promoting plant growth and yield in brinjal cultivation.

Declaration

The author declares that there is no conflict of interest.

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References

- Kaur T, Devi R, Negi R, Kumar S, Singh S, Rustagi S, *et al.* Microbial consortium with multifunctional attributes for plant growth of eggplant (*Solanum melongena* L.). *Folia Microbiol.* 2024;69:1-12.
- Shahwar D, Mushtaq Z, Mushtaq H, Alqarawi AA, Park Y, Alshahrani TS, *et al.* Role of microbial inoculants as biofertilizers for improving crop productivity: A review. *Heliyon.* 2023;9(6):e****.
- Kucey RMN, Janzen HH, Leggett ME. Microbially mediated increases in plant-available phosphorus. *Adv Agron.* 1989;42:199-228.
- Mohamed EA, Farag AG, Youssef SA. Phosphate solubilization by *Bacillus subtilis* and *Serratia marcescens* isolated from tomato rhizosphere. *J Environ Prot.* 2018;9(3):266-273.
- Jha Y. Potassium mobilizing bacteria enhance potassium uptake in paddy under salinity stress. *Braz J Biol Sci.* 2017;4(8):e282-e282.
- Deshmukh AJ, Jaiman RS, Bambharolia RP, Patil VA. Seed biopriming: A review. *Int J Econ Plants.* 2020;7(1):38-43.
- Aneja KR. Experiments in microbiology, plant pathology and biotechnology. New Delhi: New Age Int Ltd; 2003. p. 248-253.
- Pal P, Khatun N, Banerjee SK. Potential screening of *Azotobacter* from soil. *Int J Adv Res.* 2017;5(3):24-31.
- De Vos P, Garrity GM. *Bergey's manual of systematic bacteriology.* New York: Springer; 2009.
- Cappuccino JG, Sherman N. *Microbiology: A laboratory manual.* San Francisco: Pearson/Benjamin Cummings; 2005. p. 507.
- Rosemary O, Gloria G, Cecilia I. Isolation and characterization of nitrogen-fixing bacteria in soil. *Int J Life Sci Pharma Res.* 2013;2(3):1-5.
- Upadhyay S, Kumar N, Singh VK, Singh A. Isolation, characterization and morphological study of *Azotobacter* isolates. *J Appl Nat Sci.* 2015;7(2):984-990.
- Muthukumar A, Sandhya GM, Dakshayini G. Morphological and biochemical characterization of plant growth promoting microorganisms. *Int J Curr Microbiol Appl Sci.* 2021;10(2):867-874.
- Parunandi SS, Murumkar DR, Navale AM, Shelke SR. Consortial role of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria in wheat (*Triticum aestivum*). *Int J Curr Microbiol Appl Sci.* 2023;12(11):138-167.
- Yeole RD, Dube HC. Siderophore-mediated antibiosis of rhizobacteria against soil-borne fungal pathogens. *J Mycol Plant Pathol.* 2000;30(3):335-338.
- Jackson ML. *Soil chemical analysis.* 2nd ed. New Delhi: Prentice-Hall of India Pvt Ltd; 1973.
- Parmar KB. Isolation, characterization and efficacy of potash solubilizing bacteria in maize (*Zea mays* L.) [PhD thesis]. Navsari: Navsari Agricultural University; 2016.
- Jain D, Kaur G, Bhojiya AA, Chauhan S, Khandelwal SK, Meena RH, *et al.* Phenetic characterization of nitrogen-fixing *Azotobacter* from rhizospheric soil. *J Pure Appl Microbiol.* 2021;15(1):428-436.
- Priya MS, Farooq R, Divyashree K, Babu AS, Prabha ML, Prasad MP. Pilot-scale production of *Azotobacter* biofertilizer and its effect on *Ocimum sanctum*. *Int J Eng Adv Technol.* 2013;2(4):534-537.
- Sharma T, Kumar N, Rai N. Effect of nitrogen-fixing and phosphate-solubilizing bacteria on seed germination of brinjal (*Solanum melongena* L.). *J Graphic Era Univ.* 2018;7-19.
- Samal DPK, Ray P, Sukla LB, Shukla V. Isolation and screening of *Azotobacter* spp. for plant growth promoting properties. *Biointerface Res Appl Chem.* 2020;10(2):5188-5192.
- Lolita JC, Keshamma E, Shivashankrappa LH, Kavitha KR. Isolation and identification of bacteria from rhizosphere of brinjal (*Solanum melongena* L.). *Int Adv Res J Sci Eng Technol.* 2022;9(3):1-5.
- Amri M, Rjeibi MR, Gatrouti M, Mateus DM, Asses N, Pinho HJ, *et al.* Isolation and characterization of phosphate-solubilizing bacteria from soils. *Microorganisms.* 2023;11(3):783.
- Mengesha AS, Legesse NH. Isolation of phosphate solubilizing bacteria from lentil rhizosphere. *PLoS One.* 2024;19(11):e0308915.
- Hu X, Chen J, Guo J. Phosphate and potassium solubilizing bacteria from Zhejiang, China. *World J Microbiol Biotechnol.* 2006;22(9):983-990.
- Jiménez DJ, Montaña JS, Martínez MM. Characterization of nitrogen-fixing *Azotobacter* in Colombian soils. *Braz J Microbiol.* 2011;42:846-858.
- Saini M, Saini P, Chaudhary A, Chitranshi R, Dutta R. Biochemical analysis of *Bacillus subtilis* from paddy field. *Int J Chem Biol Sci.* 2025;7(2):41-45.
- Thuvarka R, Nawaratna KK, Aruggoda AGB, Kohombange S. Effect of potassium solubilizing bacteria on growth and yield of brinjal (*Solanum melongena* L.). 2023.