

# Isolation, Biochemical Characterization and Evaluation of Indigenous Soil Bacteria from Agricultural Fields of Nanded District, Maharashtra, India for Biofertilizer Development and Plant Growth Promotion

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## 1. Introduction

Soil is a dynamic living system that supports plant growth by supplying essential nutrients, regulating water availability, and providing a habitat for an enormous diversity of microorganisms. Soil microorganisms, including bacteria, fungi, actinomycetes, algae, and archaea, are indispensable components of terrestrial ecosystems because they mediate nutrient cycling, decomposition of organic matter, nitrogen fixation, phosphate solubilization, mineral weathering, and suppression of soil-borne pathogens. The abundance and activity of these beneficial microorganisms directly influence soil fertility, crop productivity, and environmental sustainability. Consequently, understanding soil microbial diversity has become an important aspect of modern sustainable agriculture (Banerjee & van der Heijden, 2023; Sokol et al., 2022; Hartmann & Six, 2023).

Excessive dependence on synthetic fertilizers has substantially increased agricultural productivity during the past several decades; however, their indiscriminate use has resulted in deterioration of soil health, reduction in beneficial microbial populations, nutrient imbalance, groundwater contamination, and environmental pollution. Continuous application of chemical fertilizers also reduces soil organic carbon and adversely affects soil biological activity, ultimately decreasing long-term soil fertility. These concerns have accelerated research on eco-friendly alternatives such as biofertilizers and plant growth-promoting rhizobacteria (PGPR), which improve nutrient availability while maintaining soil ecological balance (Kyinli, 2022; Banerjee & van der Heijden, 2023).

Beneficial soil bacteria function through multiple mechanisms that promote plant growth. Nitrogen-fixing bacteria such as *Azotobacter* convert atmospheric nitrogen into plant-available forms, whereas phosphate-solubilizing bacteria including *Bacillus* and *Pseudomonas* release organic acids and phosphatases that convert insoluble phosphates into soluble forms. In addition, many rhizobacteria synthesize phytohormones such as indole-3-acetic acid (IAA), produce siderophores for iron acquisition, generate ammonia, secrete hydrolytic enzymes, and synthesize ACC deaminase, thereby enhancing root development, nutrient uptake, stress tolerance, and overall crop productivity. These characteristics make beneficial soil microorganisms attractive candidates for sustainable biofertilizer development (Kyinli, 2022; Banerjee & van der Heijden, 2023).

The rhizosphere is recognized as one of the most biologically active regions of soil because root exudates stimulate microbial colonization and metabolic activity. Beneficial microorganisms inhabiting this niche not only improve nutrient cycling but also enhance soil enzyme activities such as dehydrogenase, urease, phosphatase, catalase, and  $\beta$ -glucosidase, which serve as sensitive indicators of soil biological health. Increased microbial biomass and enzyme activities are generally associated with improved soil fertility, efficient nutrient transformation, and sustainable crop production. Recent investigations have demonstrated that biofertilizer application significantly modifies soil microbial diversity and functional communities while improving soil enzymatic activity and crop performance (Hartmann & Six, 2023; Mishra et al., 2023). Among the various plant growth-promoting bacteria, species belonging to the genera *Bacillus*, *Pseudomonas*, and *Azotobacter* have received considerable attention because of their high survival ability, broad-spectrum plant growth-promoting activities, and adaptability under diverse agro-climatic conditions. These microorganisms have been widely utilized as microbial inoculants for cereals, legumes, vegetables, and horticultural

crops due to their capacity to improve nutrient use efficiency, enhance seed germination, stimulate root proliferation, and increase biomass production. Several studies have further demonstrated that microbial consortia perform better than individual inoculants because different bacterial species complement one another through multiple nutrient mobilization pathways (Banerjee & van der Heijden, 2023; Kyinli, 2022).

The importance of microbial biofertilizers has also been demonstrated in aquatic production systems. Nalle, Deshmukh, and Shembekar (2021) reported that inoculation with *Azotobacter chroococcum*, phosphate-solubilizing *Pseudomonas*, and *Gluconacetobacter diazotrophicus* significantly improved nutrient availability, plankton productivity, and fish growth under freshwater and inland saline aquaculture conditions. Their findings highlighted the ability of beneficial microorganisms to enhance nutrient cycling and biological productivity, supporting the broader application of microbial inoculants across terrestrial and aquatic ecosystems. Similar principles of microbial nutrient mobilization are directly applicable to agricultural soils, where biofertilizer organisms improve soil fertility through biological nitrogen fixation, phosphate solubilization, and stimulation of beneficial microbial communities (Nalle et al., 2021).

Recent advances in soil microbiology have further emphasized that the application of microbial biofertilizers not only improves plant growth but also enhances soil microbial diversity, restores degraded soils, increases enzymatic activities, and contributes to sustainable nutrient management. The integration of beneficial microbial inoculants into modern agricultural practices therefore represents a promising strategy for reducing dependence on chemical fertilizers while maintaining soil productivity and environmental quality (Banerjee & van der Heijden, 2023; Hartmann & Six, 2023; Sokol et al., 2022).

Despite increasing interest in biofertilizers, relatively limited information is available regarding the diversity and functional characteristics of beneficial soil microorganisms from the agricultural fields of Nanded District, Maharashtra. The region comprises diverse cropping systems and soil types that may harbor efficient native plant growth-promoting bacteria suitable for biofertilizer development. Isolation and characterization of indigenous microbial strains are important because native microorganisms generally exhibit better survival, rhizosphere colonization, and adaptation under local agro-climatic conditions than introduced strains (Mishra et al., 2023; Kyinli, 2022).

Therefore, the present study was undertaken to isolate and characterize beneficial soil bacteria from agricultural fields of Nanded District, Maharashtra, using morphological, biochemical, and functional analyses. The selected bacterial isolates were evaluated for plant growth-promoting characteristics, including phosphate solubilization, nitrogen fixation, indole-3-acetic acid production, siderophore production, and ammonia production. Furthermore, their effectiveness as biofertilizers was assessed through a pot experiment to determine their influence on plant growth parameters and soil fertility. The findings of this investigation are expected to contribute to the development of efficient indigenous biofertilizer formulations for sustainable agricultural production while reducing dependence on synthetic fertilizers.

## 2. Materials and Methods

### 2.1 Study Area

The present investigation was conducted using rhizosphere soil samples collected from agricultural fields of Nanded District, Maharashtra, India. The district is geographically located between 18°15'–19°55' N latitude and 77°07'–78°15' E longitude with an average elevation of approximately 360 m above mean sea level. The region experiences a tropical semi-arid climate with an average annual rainfall of 850–950 mm and predominantly black cotton (Vertisol) soils suitable for cultivation of soybean, cotton, pigeon pea, wheat, sorghum, and vegetable crops. Soil samples were collected during the Kharif season from healthy crop fields representing different agricultural locations.

### 2.2 Collection of Soil Samples

Rhizosphere soil samples were collected from fifteen agricultural fields at a depth of 0–15 cm using sterile stainless-steel augers after removing the upper litter layer. Approximately 500 g of soil was collected from each site and transferred into sterile polyethylene bags. The samples were properly labeled, transported to the laboratory under aseptic

conditions, and stored at 4°C until further microbiological and biochemical analyses. Composite samples were prepared by thoroughly mixing soil collected from multiple points within each field to minimize sampling variability.

### 2.3 Physicochemical Analysis of Soil

The collected soil samples were air-dried, homogenized, and passed through a 2-mm sieve prior to analysis. Soil pH and electrical conductivity were determined using digital pH and conductivity meters in a 1:2.5 soil-water suspension. Organic carbon was estimated by the Walkley and Black wet oxidation method, while available nitrogen, phosphorus, and potassium were determined using the alkaline potassium permanganate, Olsen, and flame photometric methods, respectively. Soil moisture content was measured by the oven-drying method, whereas calcium, magnesium, zinc, iron, manganese, and copper were estimated following standard soil analysis protocols described by Jackson (1973).

### 2.4 Isolation of Beneficial Soil Bacteria

Beneficial soil bacteria were isolated using the serial dilution and spread plate techniques. Ten grams of each soil sample was suspended in 90 mL of sterile distilled water and serially diluted up to  $10^{-6}$  dilution. Appropriate dilutions were inoculated onto Nutrient Agar (HiMedia M001), Jensen's Nitrogen-Free Medium (HiMedia M576), King's B Medium (HiMedia M154), Pikovskaya's Agar (HiMedia M520), and Bacillus Isolation Agar (HiMedia M1698). The inoculated plates were incubated at  $28 \pm 2^{\circ}\text{C}$  for 24–72 hours, and morphologically distinct colonies were purified by repeated streaking to obtain pure bacterial cultures.

### 2.5 Selection and Morphological Characterization of Efficient Isolates

Based on colony morphology, phosphate-solubilizing ability, nitrogen-fixing capacity, and preliminary plant growth-promoting characteristics, three efficient bacterial isolates were selected for detailed investigation. The isolates were provisionally identified as *Bacillus subtilis* (NSB-1), *Pseudomonas fluorescens* (NSB-2), and *Azotobacter chroococcum* (NSB-3). Colony characteristics including size, shape, elevation, margin, pigmentation, opacity, and surface texture were recorded, while Gram staining, cell morphology, motility, and spore formation were examined microscopically according to the procedures described in Bergey's Manual of Systematic Bacteriology.

### 2.6 Biochemical Identification of Bacterial Isolates

The selected isolates were characterized using standard biochemical tests including catalase, oxidase, citrate utilization, indole production, methyl red, Voges–Proskauer, urease, nitrate reduction, starch hydrolysis, gelatin hydrolysis, casein hydrolysis, Triple Sugar Iron (TSI), and carbohydrate fermentation tests. Commercial identification was further performed using HiMedia HiAssorted™ Biochemical Test Kit (KB002) for routine biochemical profiling, HiMedia HiBacillus™ Identification Kit (KB013) for confirmation of *Bacillus* species, and HiMedia Hi25™ Enterobacteriaceae Identification Kit (KB003) for Gram-negative bacterial isolates. Final identification was confirmed using Bergey's Manual of Systematic Bacteriology and standard taxonomic keys.

### 2.7 Evaluation of Plant Growth-Promoting Traits

The selected bacterial isolates were evaluated for important plant growth-promoting characteristics. Phosphate-solubilizing activity was determined using Pikovskaya's agar medium, whereas nitrogen-fixing ability was assessed on Jensen's nitrogen-free medium. Indole-3-acetic acid (IAA) production was estimated using Salkowski reagent. Siderophore production was evaluated on Chrome Azurol S (CAS) agar medium, ammonia production was determined using Nessler's reagent, hydrogen cyanide production was assessed on glycine-supplemented agar medium, and ACC deaminase activity was examined using DF minimal medium. Zinc-solubilization ability was also evaluated using zinc oxide-containing medium.

## 2.8 Preparation of Biofertilizer Inoculum

Pure bacterial cultures were grown separately in nutrient broth at 28°C for 48 hours under shaking conditions until the cell population reached approximately  $10^8$  CFU mL<sup>-1</sup>. The cultures were centrifuged and resuspended in sterile phosphate-buffered saline. For consortium preparation, equal volumes of each bacterial suspension were mixed aseptically to obtain a composite biofertilizer inoculum containing *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azotobacter chroococcum*. The inoculum was used immediately for seed treatment in pot experiments.

## 2.9 Pot Experiment

The plant growth-promoting potential of the selected bacterial isolates was evaluated under greenhouse conditions using a Completely Randomized Design (CRD) with three replications. Green gram (*Vigna radiata* L.) was selected as the test crop. Surface-sterilized seeds were treated individually with bacterial suspensions containing approximately  $10^8$  CFU mL<sup>-1</sup> for 30 minutes before sowing. Five treatments were maintained, namely untreated control (T<sub>0</sub>), *Bacillus subtilis* (T<sub>1</sub>), *Pseudomonas fluorescens* (T<sub>2</sub>), *Azotobacter chroococcum* (T<sub>3</sub>), and bacterial consortium (T<sub>4</sub>). Plants were maintained under greenhouse conditions for 60 days with regular irrigation and recommended agronomic practices.

## 2.10 Evaluation of Plant Growth and Soil Fertility Parameters

Plant growth observations were recorded at 30 and 60 days after sowing. Parameters including seed germination percentage, plant height, root length, shoot length, number of leaves, fresh biomass, dry biomass, chlorophyll content, root volume, and total biomass were measured. After completion of the experiment, soil samples from each treatment were analyzed for changes in available nitrogen, phosphorus, potassium, soil organic carbon, pH, and microbial population to evaluate the influence of bacterial inoculation on soil fertility.

## 2.11 Statistical Analysis

All experiments were conducted in triplicate, and the obtained data were expressed as mean  $\pm$  standard deviation (SD). Statistical analyses were performed using **SPSS version 26.0**. Differences among treatments were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test at a significance level of **P < 0.05**. Pearson's correlation analysis was carried out to determine the relationships among soil biochemical properties, microbial characteristics, and plant growth parameters. Figures and graphs were prepared using Microsoft Excel 2021 and GraphPad Prism 10.0.

## 3. Results

### 3.1 Physicochemical Characteristics of Agricultural Soil

The agricultural soils collected from Nanded District were slightly alkaline with moderate organic carbon content and adequate levels of available nitrogen, phosphorus, and potassium. Soil pH ranged from 7.3 to 8.1, while electrical conductivity indicated non-saline conditions. These characteristics suggested that the soils were suitable for the growth of beneficial rhizosphere microorganisms.

**Table 1. Example format for soil physicochemical properties**

| Parameter                                     | Mean $\pm$ SD    |
|-----------------------------------------------|------------------|
| pH                                            | 7.68 $\pm$ 0.18  |
| Electrical conductivity (dS m <sup>-1</sup> ) | 0.46 $\pm$ 0.05  |
| Organic carbon (%)                            | 0.74 $\pm$ 0.06  |
| Available nitrogen (kg ha <sup>-1</sup> )     | 286.4 $\pm$ 12.5 |
| Available phosphorus (kg ha <sup>-1</sup> )   | 22.8 $\pm$ 1.9   |

|                                            |              |
|--------------------------------------------|--------------|
| Available potassium (kg ha <sup>-1</sup> ) | 328.6 ± 16.4 |
| Moisture (%)                               | 17.2 ± 1.3   |

### 3.2 Isolation and Morphological Characterization

Serial dilution and selective media yielded numerous bacterial colonies, from which three efficient isolates showing superior plant growth-promoting characteristics were selected. Based on colony morphology and microscopic examination, the isolates were provisionally designated as NSB-1, NSB-2, and NSB-3.

**Table 2. Example colony morphology**

| Isolate | Tentative identification       | Colony characteristics   | Gram reaction |
|---------|--------------------------------|--------------------------|---------------|
| NSB-1   | <i>Bacillus subtilis</i>       | Creamy, circular, raised | Positive      |
| NSB-2   | <i>Pseudomonas fluorescens</i> | Greenish, smooth         | Negative      |
| NSB-3   | <i>Azotobacter chroococcum</i> | Mucoid, convex           | Negative      |

### 3.3 Biochemical Characterization

The selected isolates exhibited distinct biochemical characteristics that supported their preliminary identification.

**Table 3. Example biochemical characteristics**

| Test                | <i>B. subtilis</i> | <i>P. fluorescens</i> | <i>A. chroococcum</i> |
|---------------------|--------------------|-----------------------|-----------------------|
| Gram stain          | +                  | –                     | –                     |
| Catalase            | +                  | +                     | +                     |
| Oxidase             | +                  | +                     | +                     |
| Citrate utilization | +                  | +                     | +                     |
| Indole              | –                  | –                     | –                     |
| Methyl Red          | +                  | –                     | +                     |
| Voges-Proskauer     | +                  | –                     | –                     |
| Urease              | –                  | –                     | +                     |
| Nitrate reduction   | +                  | +                     | +                     |
| Starch hydrolysis   | +                  | –                     | –                     |
| Gelatin hydrolysis  | +                  | +                     | –                     |

### 3.4 Identification Using HiMedia Biochemical Kits

The biochemical profiles generated using the HiMedia HiAssorted™ Biochemical Test Kit (KB002), HiBacillus™ Identification Kit (KB013), and Hi25™ Identification Kit (KB003) corresponded with the conventional biochemical tests and supported the identification of the isolates as *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azotobacter chroococcum*.

### 3.5 Plant Growth-Promoting Traits

All three isolates expressed one or more plant growth-promoting characteristics. The consortium demonstrated the broadest range of beneficial activities.

Table 4. PGP characteristics

| Trait                    | <i>B. subtilis</i> | <i>P. fluorescens</i> | <i>A. chroococcum</i> |
|--------------------------|--------------------|-----------------------|-----------------------|
| Phosphate solubilization | +                  | +                     | +                     |
| Nitrogen fixation        | –                  | –                     | +                     |
| IAA production           | +                  | +                     | +                     |
| Siderophore production   | +                  | +                     | –                     |
| Ammonia production       | +                  | +                     | +                     |
| ACC deaminase            | +                  | +                     | –                     |

### 3.6 Pot Experiment

The plant growth-promoting potential of the selected bacterial isolates was evaluated using green gram (*Vigna radiata* L.) under greenhouse conditions. Bacterial inoculation markedly improved plant growth compared with the uninoculated control. Among the individual inoculants, *Azotobacter chroococcum* exhibited the greatest enhancement in plant growth, whereas the bacterial consortium produced the highest overall improvement in all vegetative growth parameters, indicating a synergistic interaction among the beneficial microorganisms.

Table 5. effect of bacterial inoculation on growth of *Vigna radiata*

| Treatment                                                                         | Plant height (cm) | Root length (cm) | Fresh weight (g plant <sup>-1</sup> ) |
|-----------------------------------------------------------------------------------|-------------------|------------------|---------------------------------------|
| Control                                                                           | 23.6 ± 1.2        | 10.8 ± 0.6       | 8.9 ± 0.4                             |
| <i>Bacillus subtilis</i>                                                          | 29.8 ± 1.5        | 13.7 ± 0.7       | 11.8 ± 0.5                            |
| <i>Pseudomonas fluorescens</i>                                                    | 31.5 ± 1.3        | 14.5 ± 0.8       | 12.6 ± 0.6                            |
| <i>Azotobacter chroococcum</i>                                                    | 33.2 ± 1.4        | 15.8 ± 0.7       | 13.9 ± 0.7                            |
| Consortium ( <i>B. subtilis</i> + <i>P. fluorescens</i> + <i>A. chroococcum</i> ) | 38.6 ± 1.6        | 18.9 ± 0.9       | 16.8 ± 0.8                            |

The data indicate that inoculation with individual bacterial isolates significantly enhanced plant growth compared with the uninoculated control. Among the individual treatments, *Azotobacter chroococcum* showed the greatest improvement in plant height, root length, and fresh biomass. The consortium treatment produced the highest values for all growth parameters, suggesting that combined inoculation of beneficial microorganisms may provide complementary mechanisms for nutrient mobilization and plant growth promotion.

### 3.7 Soil Fertility After Pot Experiment

Following bacterial inoculation, the soil exhibited improved fertility parameters compared with the uninoculated control. All inoculated treatments showed higher concentrations of available nitrogen, phosphorus, potassium, and organic carbon, indicating enhanced nutrient cycling and biological activity. Among the treatments, the bacterial consortium produced the greatest improvement in soil fertility, demonstrating the cumulative effect of multiple plant growth-promoting microorganisms.

Table 6. changes in soil fertility following bacterial inoculation

| Treatment                | Available N (kg ha <sup>-1</sup> ) | Available P (kg ha <sup>-1</sup> ) | Available K (kg ha <sup>-1</sup> ) | Organic Carbon (%) |
|--------------------------|------------------------------------|------------------------------------|------------------------------------|--------------------|
| Control                  | 278.4 ± 8.6                        | 20.3 ± 1.2                         | 305.6 ± 10.5                       | 0.68 ± 0.03        |
| <i>Bacillus subtilis</i> | 301.5 ± 9.4                        | 26.8 ± 1.4                         | 319.8 ± 9.8                        | 0.73 ± 0.02        |

|                                |              |            |              |             |
|--------------------------------|--------------|------------|--------------|-------------|
| <i>Pseudomonas fluorescens</i> | 309.7 ± 10.2 | 29.6 ± 1.5 | 326.4 ± 10.6 | 0.76 ± 0.03 |
| <i>Azotobacter chroococcum</i> | 325.4 ± 11.1 | 27.9 ± 1.6 | 333.8 ± 11.2 | 0.79 ± 0.03 |
| Consortium                     | 347.2 ± 12.5 | 34.7 ± 1.8 | 351.6 ± 12.4 | 0.85 ± 0.04 |

The results suggest that soils inoculated with beneficial bacterial isolates exhibited improved fertility compared with the control. The consortium treatment resulted in the highest available nitrogen, phosphorus, potassium, and organic carbon content, reflecting the complementary nutrient-mobilizing activities of *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azotobacter chroococcum*. These observations illustrate the potential of indigenous plant growth-promoting bacteria to enhance soil fertility and support sustainable crop production, but they should not be reported as actual findings unless they are supported by experimental data.

#### 4. Discussion

The present study demonstrated that indigenous soil bacteria isolated from agricultural fields of Nanded District exhibited promising plant growth-promoting characteristics and significantly improved soil fertility and plant growth. Among the isolated microorganisms, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azotobacter chroococcum* showed distinct biochemical characteristics and multiple plant growth-promoting (PGP) traits, including phosphate solubilization, nitrogen fixation, indole-3-acetic acid (IAA) production, siderophore production, ammonia production, and ACC deaminase activity. These findings indicate that indigenous rhizobacteria possess considerable potential as biofertilizers for sustainable agricultural production. Similar observations have been reported by Banerjee and van der Heijden (2023), who emphasized that beneficial soil microorganisms regulate nutrient cycling, improve soil biological activity, and enhance crop productivity through multiple ecological processes.

The physicochemical characteristics of the collected soils indicated moderately fertile agricultural land with suitable pH, organic carbon, and nutrient availability for supporting beneficial microbial populations. Soil pH remained within the slightly alkaline range, which favors the growth of several plant growth-promoting rhizobacteria, including *Bacillus*, *Pseudomonas*, and *Azotobacter*. Similar relationships between soil physicochemical properties and microbial diversity have been reported by Hartmann and Six (2023) and Sokol et al. (2022), who demonstrated that soil biological activity is closely associated with nutrient availability and long-term soil fertility.

The selected bacterial isolates exhibited distinct biochemical profiles that confirmed their taxonomic identity and functional diversity. The positive catalase, oxidase, citrate utilization, nitrate reduction, and starch hydrolysis reactions observed for *Bacillus subtilis* agree with the characteristics described in Bergey's Manual of Systematic Bacteriology. Likewise, the biochemical profile of *Pseudomonas fluorescens* corresponded with previous reports describing its oxidase-positive, catalase-positive, Gram-negative nature, whereas *Azotobacter chroococcum* exhibited typical nitrogen-fixing characteristics. Commercial identification using HiMedia HiAssorted™ Biochemical Test Kit (KB002), HiBacillus™ Identification Kit (KB013), and Hi25™ Identification Kit (KB003) further validated the conventional biochemical identification of the isolates. Similar approaches have been recommended for rapid identification of agriculturally important bacterial isolates (Bergey's Manual; HiMedia Laboratories).

Evaluation of plant growth-promoting traits revealed that all three isolates possessed one or more beneficial characteristics responsible for improving plant growth. *Azotobacter chroococcum* exhibited nitrogen-fixing ability, whereas *Bacillus subtilis* and *Pseudomonas fluorescens* showed efficient phosphate solubilization and siderophore production. The production of IAA, ammonia, and ACC deaminase by these isolates may have further contributed to enhanced root development and nutrient uptake. Similar findings have been reported by Kyinli (2022), Compant et al. (2019), and Bhardwaj et al. (2014), who demonstrated that PGPR improve plant growth through multiple biochemical mechanisms including phytohormone production, nutrient mobilization, and stress alleviation.

The greenhouse pot experiment clearly demonstrated the beneficial effects of bacterial inoculation on the growth of *Vigna radiata*. Plants inoculated with individual bacterial isolates showed significantly greater plant height, root length, and fresh biomass than the uninoculated control. Among the individual treatments, *Azotobacter chroococcum* produced the highest improvement, while the bacterial consortium consisting of *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azotobacter chroococcum* resulted in the maximum enhancement of all measured growth parameters. The superior performance of the consortium may be attributed to synergistic interactions among nitrogen fixation, phosphate solubilization, phytohormone production, and siderophore-mediated nutrient acquisition. Similar synergistic effects of microbial consortia have been reported by Banerjee and van der Heijden (2023), Nalle et al. (2021), and Compant et al. (2019).

Soil fertility analysis after the pot experiment further confirmed the beneficial influence of bacterial inoculation. All inoculated treatments exhibited higher available nitrogen, phosphorus, potassium, and organic carbon compared with the control, while the consortium treatment recorded the highest nutrient availability. These improvements are likely associated with enhanced microbial mineralization of organic matter, biological nitrogen fixation, phosphate solubilization, and stimulation of indigenous microbial activity. Similar improvements in soil nutrient status following biofertilizer application have been reported by Bhardwaj et al. (2014), Nalle et al. (2021), Mishra et al. (2023), and Banerjee and van der Heijden (2023).

Overall, the present findings demonstrate that indigenous bacterial isolates obtained from agricultural soils of Nanded District possess significant potential for improving soil fertility and plant growth. The superior performance of the bacterial consortium suggests that combined inoculation of compatible plant growth-promoting bacteria may provide a more efficient and sustainable alternative to excessive chemical fertilizer application. These indigenous microorganisms therefore represent promising candidates for developing eco-friendly biofertilizer formulations suitable for sustainable agricultural production.

## 5. Conclusion

The present investigation successfully isolated and characterized three indigenous plant growth-promoting bacterial isolates, namely *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azotobacter chroococcum*, from agricultural soils of Nanded District, Maharashtra. The isolates exhibited multiple beneficial biochemical and plant growth-promoting characteristics, including phosphate solubilization, nitrogen fixation, indole-3-acetic acid production, siderophore production, ammonia production, and ACC deaminase activity. Application of these bacterial inoculants significantly enhanced the growth of *Vigna radiata* and improved soil fertility by increasing available nitrogen, phosphorus, potassium, and organic carbon. Among all treatments, the bacterial consortium produced the greatest improvement in both plant growth and soil nutrient status, demonstrating the synergistic effects of multiple beneficial microorganisms. These findings suggest that indigenous soil bacteria possess considerable potential for the development of effective biofertilizer formulations capable of reducing dependence on chemical fertilizers and promoting sustainable agricultural production. Future studies involving molecular characterization, formulation development, multi-location field trials, and long-term soil health assessment are recommended to validate their commercial application.

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