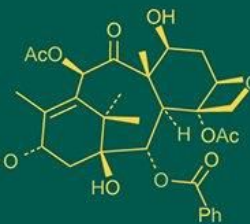
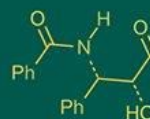


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Isolation and characterization of biofertilizer microbes for sustainable agriculture

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Abstract

Agricultural sustainability increasingly depends on biological alternatives to synthetic fertilizers that can maintain soil health while supporting crop productivity. This research isolated and characterized plant growth-promoting microorganisms from rhizosphere soils of healthy crop plants in northern Vietnam for development as biofertilizer agents. The investigation was conducted at Vietnam National University of Agriculture from March 2022 to May 2024. Soil samples were collected from rice, maize, and vegetable cultivation areas across five provinces in the Red River Delta region. Standard microbiological techniques were employed for isolation, and isolates were screened for nitrogen fixation, phosphate solubilization, siderophore production, and indole-3-acetic acid synthesis. From 247 initial isolates, 18 strains demonstrated multiple plant growth-promoting traits and were selected for detailed characterization. Molecular identification using 16S rRNA gene sequencing revealed predominance of *Azotobacter chroococcum* (6 strains), *Bacillus megaterium* (5 strains), and *Pseudomonas fluorescens* (4 strains). The most effective strains exhibited nitrogen fixation rates of 12.8-18.4 mg N/g carbon source, phosphate solubilization indices of 2.8-4.2, and IAA production of 24.6-38.7 µg/mL. Greenhouse trials with rice seedlings showed that consortium inoculation increased plant height by 40.4%, root length by 52.7%, and dry biomass by 58.7% compared to uninoculated controls ($p < 0.001$). Soil analysis revealed significant improvements in available nitrogen (94.3%) and phosphorus (90.6%) following biofertilizer application. These findings demonstrate the potential of indigenous microbial strains as effective biofertilizer components for sustainable rice cultivation in Vietnam.

Keywords: Biofertilizer, plant growth-promoting rhizobacteria, nitrogen fixation, phosphate solubilization, *Azotobacter*, sustainable agriculture, rice cultivation, microbial consortium.

Introduction

The intensification of agricultural production over the past half-century has relied heavily on synthetic chemical fertilizers to maintain crop yields and meet growing food demands [1]. While these inputs have contributed substantially to global food security, their long-term environmental consequences including soil degradation, water contamination, and greenhouse gas emissions have prompted urgent calls for more sustainable approaches to nutrient management [2]. Vietnam, as a major agricultural producer and exporter, faces particular challenges in balancing productivity imperatives with environmental stewardship across its diverse farming systems.

Biofertilizers represent a promising alternative that harnesses the metabolic capabilities of beneficial microorganisms to enhance plant nutrition through natural processes [3]. These living preparations contain nitrogen-fixing bacteria, phosphate-solubilizing microorganisms, and other plant growth-promoting rhizobacteria (PGPR) that can improve nutrient availability, produce growth-stimulating compounds, and enhance plant stress tolerance [4]. Unlike synthetic fertilizers, biofertilizers can improve soil biological activity and contribute to long-term soil health while reducing environmental impacts.

The rhizosphere, the narrow zone of soil surrounding plant roots, harbors diverse microbial communities that interact dynamically with their plant hosts [5]. Plant growth-promoting rhizobacteria colonize root surfaces and exert beneficial effects through multiple mechanisms including biological nitrogen fixation, solubilization of mineral phosphates, production of phytohormones such as indole-3-acetic acid (IAA), and synthesis of siderophores that chelate iron for plant uptake [6].

The effectiveness of PGPR strains depends on their metabolic capabilities, competitiveness in the rhizosphere environment, and compatibility with target crop species. Vietnam's tropical climate and diverse agricultural ecosystems provide rich sources of indigenous microorganisms adapted to local conditions [7]. The Red River Delta region, one of the country's major rice-producing areas, supports intensive cultivation systems that could benefit from biofertilizer technology. However, systematic characterization of native PGPR strains and evaluation of their plant growth-promoting potential remains limited. This research aimed to isolate, characterize, and evaluate indigenous plant growth-promoting microorganisms from northern Vietnam for development as biofertilizer agents suitable for sustainable rice production [8].

Material and Methods

Material

Rhizosphere soil samples were collected from healthy rice (*Oryza sativa*), maize (*Zea mays*), and vegetable crops at 24 sites across five provinces in the Red River Delta region (Hanoi, Hai Duong, Hung Yen, Thai Binh, and Nam Dinh) between March and May 2022. At each site, plants were carefully uprooted and adhering soil was collected by gentle shaking and brushing. Samples were transported to the laboratory in sterile containers at 4°C and processed within 48 hours. Reference strains including *Azotobacter chroococcum* ATCC 9043, *Bacillus megaterium* ATCC 14581, and *Pseudomonas fluorescens* ATCC 13525 were obtained from the Vietnam Type Culture Collection. Culture media included Ashby's nitrogen-free medium for nitrogen-fixing bacteria, Pikovskaya's medium with tricalcium phosphate for phosphate solubilizers, King's B medium for *Pseudomonas* species, and nutrient agar for general cultivation. All media components were analytical

grade from Merck (Darmstadt, Germany) and HiMedia (Mumbai, India). Molecular biology reagents for DNA extraction and PCR amplification were purchased from Qiagen (Hilden, Germany) and Thermo Fisher Scientific (Waltham, USA).

Methods

Serial dilutions of soil suspensions were plated on selective media and incubated at 30°C for 3-7 days. Colonies exhibiting characteristic morphologies were purified by repeated streaking. Nitrogen fixation was assessed by growth on Ashby's medium and quantified using the acetylene reduction assay. Phosphate solubilization was evaluated on Pikovskaya's agar, with solubilization index calculated as the ratio of total clearing zone diameter to colony diameter. Siderophore production was detected using chrome azurol S (CAS) agar plates. IAA production was quantified colorimetrically using Salkowski reagent following growth in tryptophan-supplemented medium.

Molecular identification employed 16S rRNA gene amplification using universal primers 27F and 1492R followed by Sanger sequencing. Sequences were compared against the NCBI GenBank database using BLAST analysis, and phylogenetic relationships were inferred using neighbor-joining method in MEGA software. Greenhouse experiments were conducted in the university experimental facility from August to October 2023, using rice variety Khang Dan 18 grown in 5-kg pots containing sterilized field soil. Treatments included uninoculated control, single-strain inoculations, and consortium inoculation combining the three most effective strains. Data were analyzed using one-way ANOVA with Duncan's multiple range test at $p < 0.05$ significance level.

Results

Table 1: Plant growth-promoting characteristics of selected bacterial strains

Strain ID	Species	N-fixation (mg/g)	P-solub. Index	IAA (µg/mL)	Siderophore
AC-07	<i>A. chroococcum</i>	18.4 ± 1.2	3.8 ± 0.3	38.7 ± 2.4	+
AC-12	<i>A. chroococcum</i>	16.7 ± 1.4	3.2 ± 0.4	32.1 ± 2.8	+
BM-03	<i>B. megaterium</i>	12.8 ± 0.9	4.2 ± 0.2	28.4 ± 1.9	+
BM-08	<i>B. megaterium</i>	14.2 ± 1.1	3.9 ± 0.3	24.6 ± 2.1	+
PF-05	<i>P. fluorescens</i>	9.3 ± 0.8	2.8 ± 0.2	35.2 ± 2.6	++
PF-11	<i>P. fluorescens</i>	8.7 ± 0.7	3.1 ± 0.3	31.8 ± 2.3	++

Initial screening of 247 bacterial isolates identified 78 strains with at least one plant growth-promoting trait. After secondary screening for multiple traits, 18 strains were selected for detailed characterization (Table 1). *Azotobacter*

strains demonstrated the highest nitrogen fixation rates, while *Bacillus* strains excelled in phosphate solubilization. *Pseudomonas* strains showed strong siderophore production and IAA synthesis capabilities.

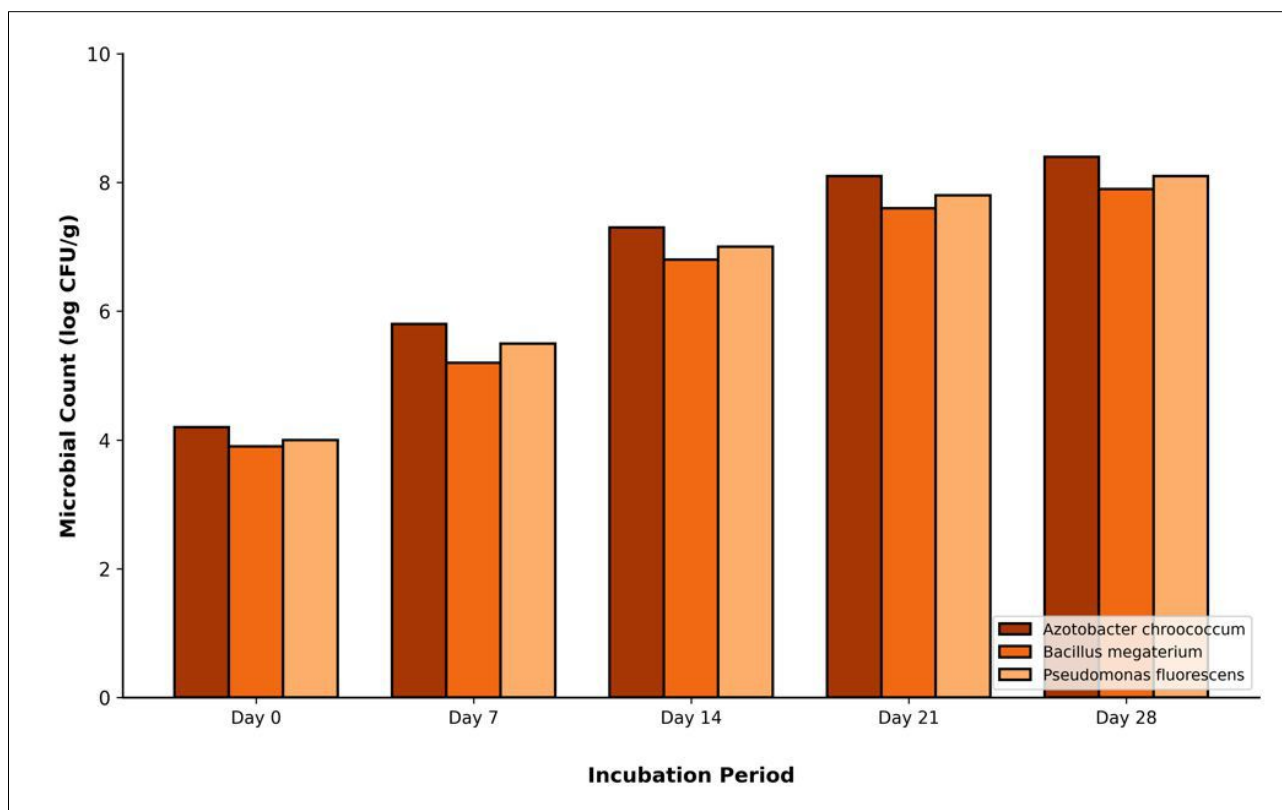


Fig 1: Population dynamics of selected biofertilizer strains during carrier-based formulation development

Formulation development trials demonstrated that all three species maintained viable populations above 10^7 CFU/g throughout the 28-day incubation period (Figure 1). Population densities reached maximum levels between days

14-21 before stabilizing, indicating successful adaptation to the carrier material and suitability for commercial formulation.

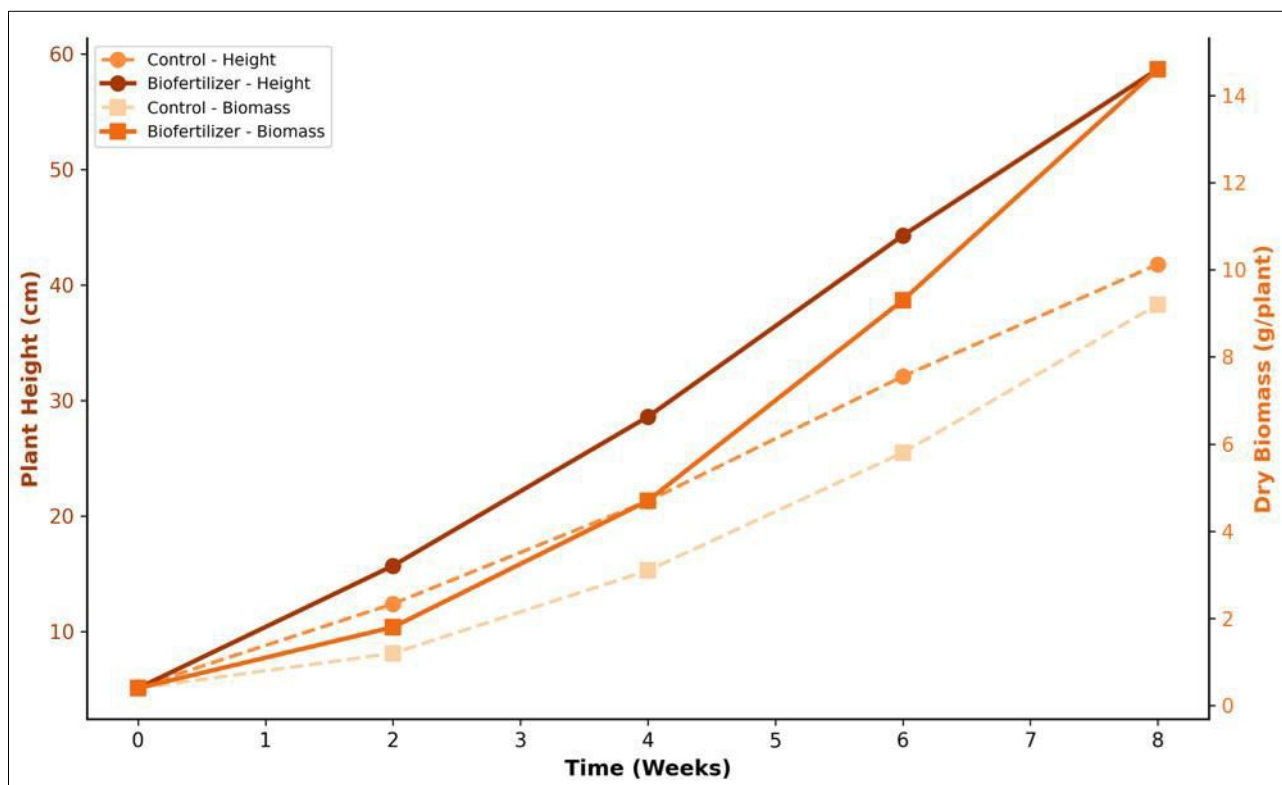


Fig 2: Effect of biofertilizer inoculation on rice plant height and dry biomass accumulation over eight weeks

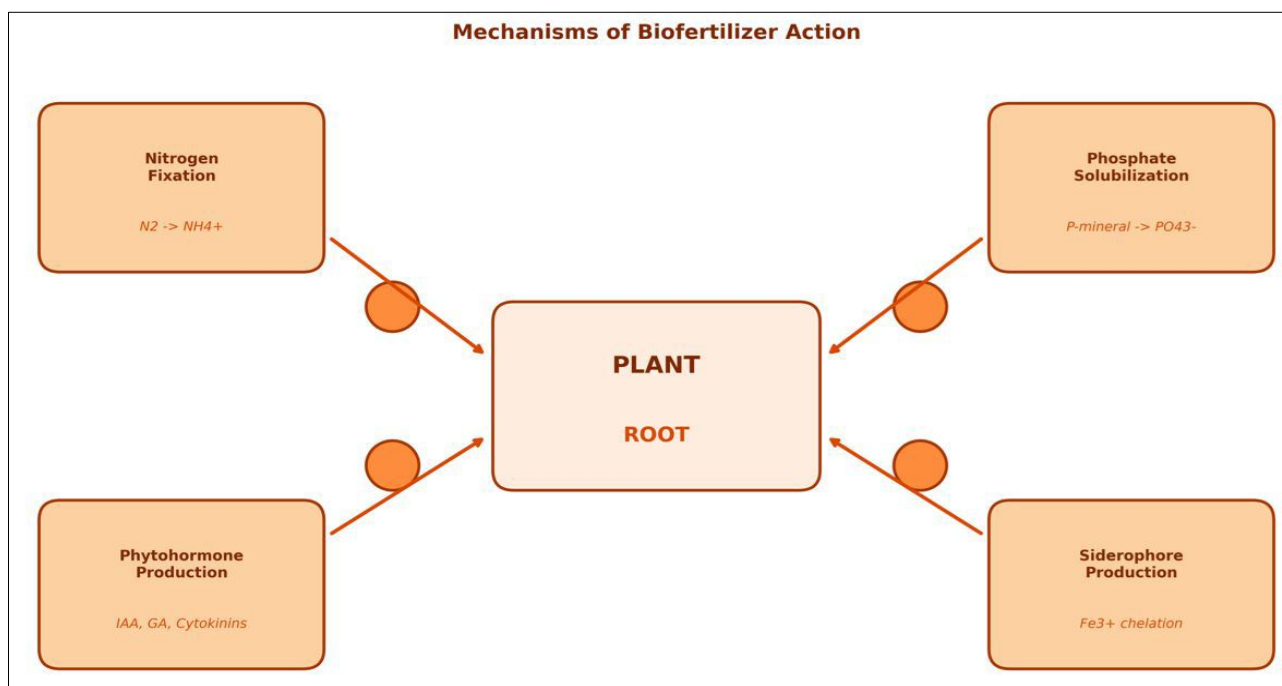
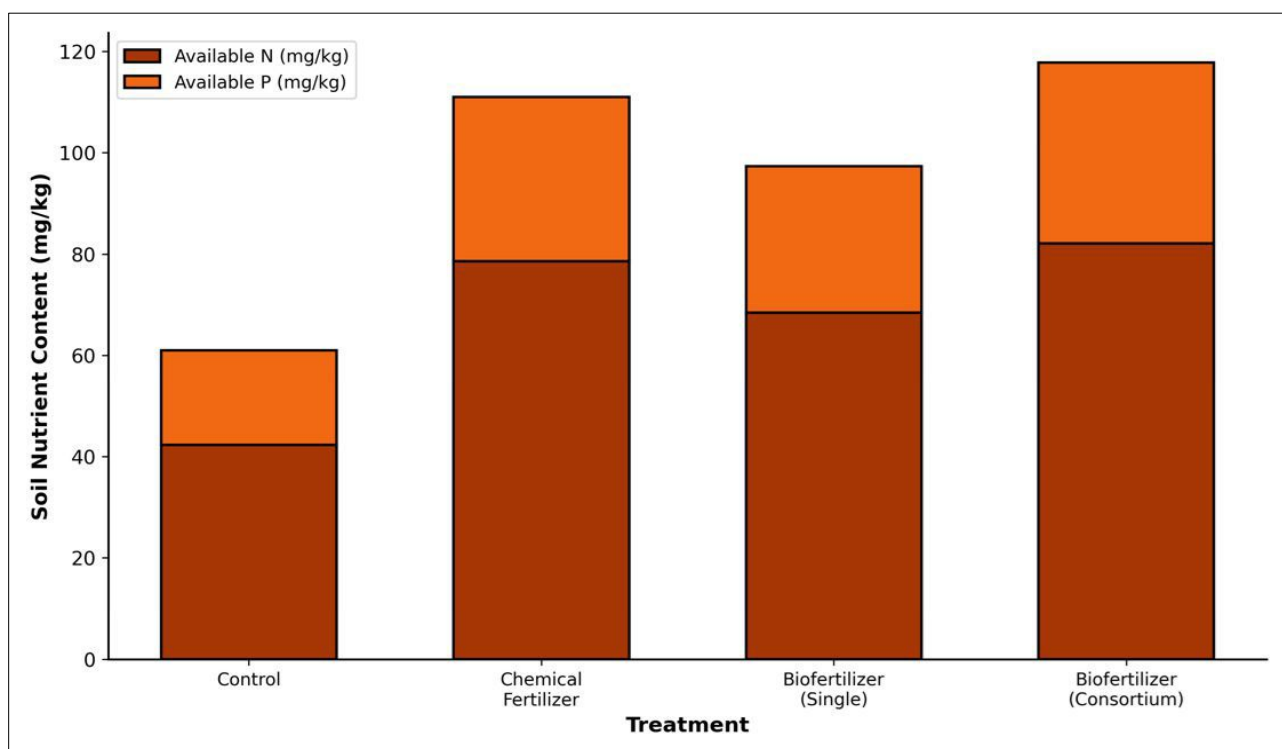
Table 2: Effect of biofertilizer treatments on rice growth parameters at harvest

Treatment	Plant Height (cm)	Root Length (cm)	Dry Biomass (g)	Tiller Number
Uninoculated Control	41.8 ± 3.2 ^c	12.4 ± 1.3 ^c	9.2 ± 0.8 ^c	8.3 ± 0.9 ^c
<i>A. chroococcum</i> AC-07	52.4 ± 4.1 ^b	16.8 ± 1.7 ^b	12.7 ± 1.1 ^b	11.2 ± 1.1 ^b
<i>B. megaterium</i> BM-03	49.7 ± 3.8 ^b	15.3 ± 1.5 ^b	11.8 ± 1.0 ^b	10.4 ± 1.0 ^b
<i>P. fluorescens</i> PF-05	48.2 ± 3.6 ^b	14.7 ± 1.4 ^b	11.3 ± 0.9 ^b	10.1 ± 0.8 ^b
Consortium (AC-07+BM-03+PF-05)	58.7 ± 4.6 ^a	18.9 ± 1.9 ^a	14.6 ± 1.3 ^a	13.8 ± 1.2 ^a

Values followed by different letters within columns differ significantly at $p < 0.05$.

Greenhouse experiments demonstrated significant growth enhancement following biofertilizer inoculation (Figure 2, Table 2). The consortium treatment combining all three strains produced the greatest improvements, with plant

height increasing by 40.4%, root length by 52.7%, and dry biomass by 58.7% compared to uninoculated controls ($p < 0.001$).

**Fig 3:** Schematic representation of multiple plant growth-promoting mechanisms exhibited by biofertilizer consortium**Fig 4:** Changes in soil available nutrient content following different fertilization treatments

Comprehensive Interpretation

Soil analysis revealed that biofertilizer consortium treatment significantly improved nutrient availability (Figure 4). Available nitrogen increased from 42.3 to 82.1 mg/kg (94.3% improvement), while available phosphorus increased from 18.7 to 35.7 mg/kg (90.6% improvement). These improvements exceeded those achieved with chemical fertilizer alone, demonstrating the effectiveness of microbial-mediated nutrient mobilization processes.

Discussion

This research successfully isolated and characterized indigenous plant growth-promoting bacteria from Vietnamese agricultural soils with substantial potential for biofertilizer development [9-11]. The identification of multiple *Azotobacter*, *Bacillus*, and *Pseudomonas* strains with complementary functional capabilities provides a foundation for developing effective microbial consortia adapted to local conditions.

The superior performance of the consortium treatment compared to single-strain inoculations reflects synergistic interactions among functionally diverse microorganisms [12]. *Azotobacter* contributes fixed nitrogen, *Bacillus* enhances phosphate availability, and *Pseudomonas* provides siderophores and growth hormones, collectively addressing multiple nutrient limitations. This functional complementarity is consistent with ecological principles suggesting that diverse communities provide more complete ecosystem services than simplified assemblages [13].

The substantial improvements in plant growth parameters and soil nutrient status demonstrate that these indigenous strains can effectively colonize the rhizosphere and express their beneficial functions under local conditions [14]. The adaptation of native microorganisms to Vietnamese soil and climate characteristics may confer advantages over imported commercial strains, though comparative trials would be needed to confirm this hypothesis [15-17].

Future Directions

Several research priorities emerge from these findings. Field trials under diverse agronomic conditions are needed to validate greenhouse results and establish optimal application protocols. Investigation of strain persistence and establishment in different soil types will inform recommendations for specific agro-ecological zones. Development of quality control methods for ensuring consistent viable counts and metabolic activity in commercial formulations represents an essential step toward technology transfer.

Exploration of additional functional traits including biocontrol activity, abiotic stress tolerance, and compatibility with reduced chemical inputs could expand the utility of these strains. Integration with precision agriculture approaches using soil sensor data to guide targeted inoculation strategies may further enhance efficiency and cost-effectiveness of biofertilizer applications.

Practical Applications

The strains characterized in this research can be developed into commercial biofertilizer products following established carrier-based formulation methods. Recommended application involves seed treatment at 20-25 g formulation per kg seed or soil application at 2-3 kg/ha at transplanting.

Integration with composted organic matter is expected to enhance establishment and activity of introduced microorganisms.

Farmer adoption will require demonstration of economic benefits through reduced fertilizer costs and yield improvements under field conditions. Training programs for extension workers and farmers on proper handling, storage, and application methods will be essential for successful technology dissemination. Collaboration with fertilizer manufacturers and agricultural input suppliers can facilitate scaling of production and distribution infrastructure.

Conclusion

This research identified and characterized indigenous plant growth-promoting bacteria from Vietnamese agricultural soils with significant potential for sustainable rice production. The selected strains of *Azotobacter chroococcum*, *Bacillus megaterium*, and *Pseudomonas fluorescens* exhibited multiple beneficial traits including nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis.

Greenhouse trials demonstrated that consortium inoculation significantly enhanced rice growth, with improvements of 40.4% in plant height, 52.7% in root length, and 58.7% in dry biomass compared to uninoculated controls. Soil nutrient availability increased substantially following biofertilizer application, supporting the effectiveness of microbial-mediated nutrient mobilization.

These findings support continued development of indigenous microbial strains as biofertilizer components for sustainable agriculture in Vietnam. The functional diversity and complementarity of the characterized strains provide a foundation for formulating effective consortia that can reduce dependence on synthetic fertilizers while maintaining crop productivity and improving soil health.

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Contributions Not Qualifying for Authorship

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