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Isolation and identification of IAA-producing rhizobacteria from Robusta coffee plantations in Dak Nong province

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***Abstract*:**

This study aimed to isolate and identify indole-3-acetic acid (IAA)-producing microorganisms from the rhizosphere of Robusta coffee plantations in Dak Nong province, Vietnam. A total of 539 microbial strains (319 bacteria, 140 fungi, and 80 actinomycetes) were isolated and screened for IAA production using the Salkowski colourimetric method. Key findings revealed that 32.0% of bacterial isolates and 33.6% of fungal isolates demonstrated IAA production above 1.0 µg/ml, with 9 bacterial strains and 4 fungal strains producing more than 50.0 µg/ml of IAA. In contrast, only 3.8% of actinomycetes showed minimal activity (1.0-10.0 µg/ml). Key findings of this study are the identification of strain DN13_B03, which exhibited the highest IAA production (178 µg/ml), confirmed by High-performance liquid chromatography (HPLC) analysis with a distinct peak matching the standard IAA sample. Based on morphological characteristics (yellowish-white colonies, Gram-positive rod-shaped cells) and *16S rRNA* gene sequencing, we identified DN13_B03 as *Priestia megaterium*. Optimisation studies revealed that maximum IAA production (205 µg/ml) was achieved with 0.5% L-tryptophan, at pH 6.0, within a broad temperature range (25-40°C). The remarkable IAA-producing capability of this indigenous strain presents significant potential for developing region-specific biofertilisers to enhance sustainable coffee cultivation in Vietnam.

Keywords: **coffee tree, Dak Nong, IAA, *Priestia megaterium*, rhizosphere.**

Classification numbers: **3.1, 3.4, 3.5**

1. Introduction

Coffee is one of Vietnam's key agricultural crops, particularly in the Central Highlands region. Dak Nong province ranks as Vietnam's third-largest Robusta coffee-growing area, with approximately 140,000 hectares under cultivation and an annual yield of about 356,000 tonnes [1]. However, coffee quality and productivity in Dak Nong and many other cultivation regions across the country are increasingly compromised by farming practices that rely heavily on inorganic fertilisers. Research into biological products to replace inorganic fertilisers is therefore of significant importance to ensure the sustainable development of Vietnam's coffee industry.

Microorganisms play crucial roles in agricultural ecosystems, participating in numerous biochemical processes that enhance soil fertility and plant growth. The rhizosphere, defined as the narrow zone of soil influenced by root secretions, harbours diverse microbial communities that significantly impact plant health and productivity. These beneficial microbes produce various metabolites, including phytohormones, enzymes, antimicrobial compounds, and volatile organic compounds, which directly and indirectly promote plant growth [2].

Recent studies have demonstrated that microbial inoculants can serve as sustainable alternatives to chemical fertilisers while improving crop resilience to environmental stresses. For instance, microbial formulations containing beneficial bacteria have shown remarkable effects on enhancing nutrient uptake, mitigating salt stress, and improving drought tolerance in various crops [3]. Additionally, secondary metabolites from soil microorganisms can function as biostimulants, significantly improving plant physiological processes such as photosynthesis, nutrient absorption, and stress responses [4].

The application of microbial-based biofertilisers in agriculture represents an environmentally friendly approach to enhancing crop production while reducing chemical inputs. Several studies have reported the successful utilisation of rhizobacteria to improve crop yield, product quality, and soil health across diverse agroecosystems [5].

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The multifunctional benefits of these microbial inoculants make them promising candidates for sustainable agricultural practices.

Plant growth-promoting rhizobacteria (PGPR) are bacteria that reside in the root zone of plants and can stimulate plant growth through various mechanisms. PGPR play a crucial role in sustainable agriculture by helping to increase crop yields while minimising the use of chemical fertilisers and pesticides. They operate through several mechanisms, including phytohormone regulation, enhancement of nutrient uptake, nitrogen fixation, phosphate solubilisation, and strengthening plant resistance against pathogenic agents [6]. Among these mechanisms, the production of plant growth hormones, particularly auxins, is one of the most significant ways in which PGPR directly influence plant development.

Indole-3-acetic acid is a natural auxin that plays a vital role in plant development. IAA is produced by many rhizobacterial species, particularly those within the genera *Pseudomonas, Bacillus, Rhizobium,* and *Azospirillum*. IAA stimulates root development and enhances the plant's ability to absorb water and nutrients, thereby improving resilience under drought and nutrient-deficient conditions. Additionally, IAA is involved in many other important physiological processes, such as photosynthesis, cell division, cell elongation, and the regulation of gene expression [7]. Coffee plants are particularly responsive to auxin-mediated root development. Furthermore, IAA-producing microorganisms have demonstrated exceptional effectiveness in mitigating drought stress - an increasingly prevalent challenge in coffee-growing regions due to climate change. Unlike mechanisms such as nitrogen fixation and phosphate solubilisation, which primarily address nutrient limitations, IAA directly modulates plant architecture and physiological processes even under optimal nutrient conditions, providing benefits throughout the plant's lifecycle. For these reasons, the study of IAA-producing microorganisms is of particular relevance to sustainable coffee cultivation.

Despite numerous studies on IAA-producing rhizobacteria in various crop species, research focusing on rhizobacteria in Robusta coffee in Vietnam, particularly in Dak Nong province, remains limited. While some studies have investigated general microbial diversity in coffee plantations [8, 9], there is a notable absence of comprehensive research specifically characterising indigenous IAA-producing bacteria from Vietnam's coffee rhizosphere and their potential applications in sustainable coffee production. Consequently, significant knowledge gaps remain regarding the diversity and potential utilisation of these bacteria in sustainable coffee farming.

Based on these gaps, we formulated several key research questions and hypotheses to guide our study. We sought to determine the prevalence and diversity of IAA-producing microorganisms in the Robusta coffee rhizosphere in Dak Nong Province, identify indigenous rhizobacteria with superior IAA production capabilities, and evaluate how environmental factors influence this production. We hypothesised that bacteria would be the predominant IAA producers compared to fungi and actinomycetes; that indigenous strains would possess unique adaptations yielding higher IAA production than previously reported strains; and that optimising environmental conditions would significantly enhance IAA yields.

To test these hypotheses, we aim to isolate and identify high IAA-producing rhizobacteria from Robusta coffee-growing areas in Dak Nong province, characterise the most promising strain using morphological and molecular techniques, and determine optimal conditions for IAA production by this strain. Our research findings will serve as a foundation for developing microbial products for sustainable coffee cultivation, thereby reducing dependence on chemical fertilisers, contributing to environmental protection, and enhancing the value of coffee production in Dak Nong province.

2. Materials and methods

2.1. Microorganism growth conditions

Bacteria were cultured on tryptic soy agar (TSA) medium (6 g/l tryptone soya broth, 50 µg/ml nystatin, pH 7.0) or Luria-Bertani (LB) medium (10 g/l tryptone, 10 g/l NaCl, 5 g/l yeast extract, pH 7.0) at 28°C for 1-3 days. Fungi were cultivated on Potato Dextrose Agar (PDA) medium (12 g/l potato dextrose broth, 100 µg/ml chloramphenicol, pH 5.6) at 28°C for 7-10 days. Actinomycetes were cultured in various media, including Gauze medium (20 g/l soluble starch, 1 g/l KNO_3, 0.5 g/l NaCl, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 0.5 g/l K_2HPO_4, 10 mg/l $FeSO_4 \cdot 7H_2O$, 25 mg/ml nalidixic acid, 50 mg/ml cycloheximide, pH 7.4) for initial isolation from soil samples; Yeast Extract-Starch (YS) medium (10 g/l soluble starch, 2 g/l yeast extract, pH 7.2) for purification and maintenance of isolated colonies; and Modified Nutrient Agar (MNA) medium (5 g/l peptone, 3 g/l yeast extract, 10 g/l NaCl, pH 7.0) for IAA production testing, all incubated at 28°C for 3-4 days. For microbial culture on solid media, 15 g/l agar was added to the medium.

2.2. Sampling and sample preservation

Soil sampling and preservation procedures were conducted in accordance with TCVN 7538-6:2010 (ISO 10381-6:2009) [10]. Rhizosphere soil samples were collected from Robusta coffee plantations at two growth stages (2-year-old and 6-year-old trees) located in Nam Da ward (Krong No district before the administrative

merger) and Thuan An ward (Dak Mil district before the administrative merger) of Dak Nong province, Vietnam. Sampling was performed at the end of the rainy season (October - November 2022).

Five soil samples were collected from each plantation using a diagonal sampling pattern. Each soil sample comprised a composite of soil collected from three points surrounding a coffee tree, arranged in an equilateral triangle with the tree trunk serving as the centre point. Samples were collected from a depth of 5-15 cm. The collected soil samples were transferred to sterile polyethylene bags and stored in the dark at 4-8°C until microbial isolation, with storage time not exceeding one month.

2.3. Isolation of rhizosphere microorganisms

A total of 40 rhizosphere soil samples were collected from coffee plantations in Dak Nong province (Vietnam). One gram of each sample was suspended in 9 ml of PBS buffer (containing 8 g/l NaCl, 0.2 g/l KCl, 1.44 g/l Na_2HPO_4, 0.24 g/l KH_2PO_4, pH 7.2). One millilitre of each suspension was serially diluted up to 10^{-6}. Then, 0.1 ml of each dilution was plated on PDA (for fungal isolation), Gauze medium (for actinomycete isolation), and TSA (for bacterial isolation). Single colonies were picked and streaked onto the same medium (for fungal and bacterial isolation) and onto YS medium (for actinomycete isolation) to obtain pure cultures. Well-isolated colonies were preserved for subsequent analysis of IAA production activity.

2.4. Determination of IAA production ability of isolated microorganism strains

The isolated microorganism strains were cultured with shaking in 5 ml of medium (PDA for fungi, MNA for actinomycetes, and LB for bacteria) supplemented with 0.5% L-tryptophan at 28°C for 7 days for fungi, 3-4 days for actinomycetes, and 2-3 days for bacteria, respectively. All experiments were performed in triplicate (n=3) using independent cultures to ensure reproducibility. Two millilitres of the culture broth were centrifuged at 12,500 rpm for 5 minutes; 100 µl of the supernatant was then transferred to a 96-well plate. Two hundred microlitres of Salkowski reagent (10 mM $FeCl_3$, 35% $HClO_4$) were added to each well, and the plate was incubated in the dark at room temperature for 30 minutes [11]. The optical density (OD) was measured at 530 nm after incubation. Negative controls comprised medium without L-tryptophan supplementation. The IAA concentration in the culture medium was determined based on a standard curve constructed using standard IAA solutions. Results are presented as mean values ± standard deviation (SD).

2.5. Quantification of IAA by HPLC analysis

A single bacterial colony was cultured with shaking in 5 ml of LB medium at 28°C overnight. Each HPLC analysis [12] was performed in triplicate (n=3) using independently prepared samples. The culture broth was centrifuged at 12,500 rpm for 5 minutes. Five microlitres of the supernatant were filtered through a 0.22 µm membrane filter and injected into a 1.8 µM reverse-phase column (Agilent ZORBAX Eclipse XDB - C18, 50×4 mm) in an Agilent 1260 Infinity II LC System. The mobile phase consisted of methanol:water:acetic acid (30:69:1, v/v/v) at a flow rate of 1.0 ml/min. IAA was detected using a UV detector at 280 nm with a total run time of 30 minutes at a column temperature of 25°C. The retention time for IAA under these conditions was approximately 18 minutes, as indicated by the major peak in the chromatogram. An LB medium solution containing 100 µg/ml IAA was used as the standard.

2.6. Bacterial identification by 16S rRNA gene sequencing

Total bacterial DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega). Fifty nanograms of genomic DNA were used as a template for PCR amplification of the *16S rRNA* gene with the primer pair 27F (5'- TAACACATGCAAGTCGAACG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [13] using an Eppendorf Mastercycler EP Gradient S system. PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Scientific) and sequenced by a DNA sequencing company (Vietnam). The sequencing results were processed using BioEdit v7.0 software and analysed using the BLAST tool. Phylogenetic trees were constructed using MAFFT v7 software [14].

2.7. Determination of optimal media composition and cultivation conditions for IAA production

A one-factor-at-a-time methodology was employed to determine the optimal tryptophan concentration, pH, and temperature conditions for maximising IAA yield. Each factor was tested independently while keeping the other parameters constant. To assess the effect of tryptophan concentration, bacteria were cultured in LB medium supplemented with 0.05, 0.1, 0.5, 1.0 and 2.0% L-tryptophan at pH 7.0 and 28°C. To evaluate the effect of pH, bacteria were cultured in LB medium supplemented with 1.0% L-tryptophan at pH values ranging from 5 to 9, maintained at 28°C. To examine the effect of temperature, bacteria were cultured in LB medium supplemented with 1.0% L-tryptophan at pH 7.0, at temperatures ranging from 25° to 40°C.

All optimisation experiments were conducted in triplicate (n=3), and results are presented as mean values ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with the significance level set at $p<0.05$, using SPSS software (version 22.0, IBM Corp., USA).

3. Results and discussion

3.1. Isolation and selection of IAA-producing microbial strains

A total of 539 microbial strains were isolated from the rhizosphere of Robusta coffee plants in Dak Nong province, comprising 319 bacterial strains, 140 fungal strains, and 80 actinomycete strains (Table 1). All isolates were screened for IAA production capability using the Salkowski colorimetric method, which produces a pink-red coloration in the presence of IAA (Fig. 1A). Among the 319 bacterial isolates, 102 strains (32.0%) demonstrated IAA production above 1.0 µg/ml, with nine strains producing more than 50.0 µg/ml (Table 1). This proportion aligns with previous studies reporting that approximately 18-40% of rhizosphere bacteria exhibit IAA production capability [15].

Table 1. Results of isolation of IAA-producing microorganisms.

Microorganisms	No. isolated strains	IAA production activity (µg/ml)			
		<1.0	*1.0-10.0*	*10.0-50.0*	*>50.0*
Bacteria	319	217	64	29	9
Fungi	140	93	29	14	4
Actinomycetes	80	77	3	0	0

For fungi, 47 out of 140 strains (33.6%) showed IAA production activity, with four strains producing over 50.0 µg/ml (Table 1). Actinomycetes exhibited the lowest IAA production capability, with only three out of 80 strains (3.8%) producing IAA in the range of 1.0-10.0 µg/ml, and none exceeding this range (Table 1). The higher proportion of IAA producers among bacteria and fungi compared to actinomycetes suggests that these groups may play more significant roles in plant growth promotion through auxin production within the coffee rhizosphere ecosystem [16].

Among all isolates, we identified the bacterial strain DN13_B03 as the most potent IAA producer. Its IAA production was confirmed by HPLC analysis, which showed a distinctive peak with a retention time matching that of the standard IAA sample (Fig. 1B). Quantitative analysis determined that strain DN13_B03 produced IAA at a concentration of 178 µg/ml under the test conditions. This production level is remarkably high compared to previous reports, where most studies on rhizobacteria have documented IAA production in the range of 10-60 µg/ml. For instance, A. Karnwal (2009) [17] reported IAA production ranging from 10 to 32 µg/ml for rhizobacteria isolated from various crops, while C. Datta, et al. (2000) [18] found maximum IAA production of 61 µg/ml from rhizosphere isolates.

The exceptionally high IAA-producing capability of strain DN13_B03 offers significant potential for applications in sustainable coffee cultivation. The effects of IAA are particularly beneficial for coffee plants in the Central Highlands of Vietnam, where they frequently face nutrient limitations and drought stress. The isolation of this indigenous high IAA-producing strain presents an opportunity to develop region-specific biofertilisers. As a native strain, DN13_B03 is likely to be better adapted to local environmental conditions and may offer superior performance compared to commercial biofertilisers derived from strains isolated in different geographical regions or from different host plants.

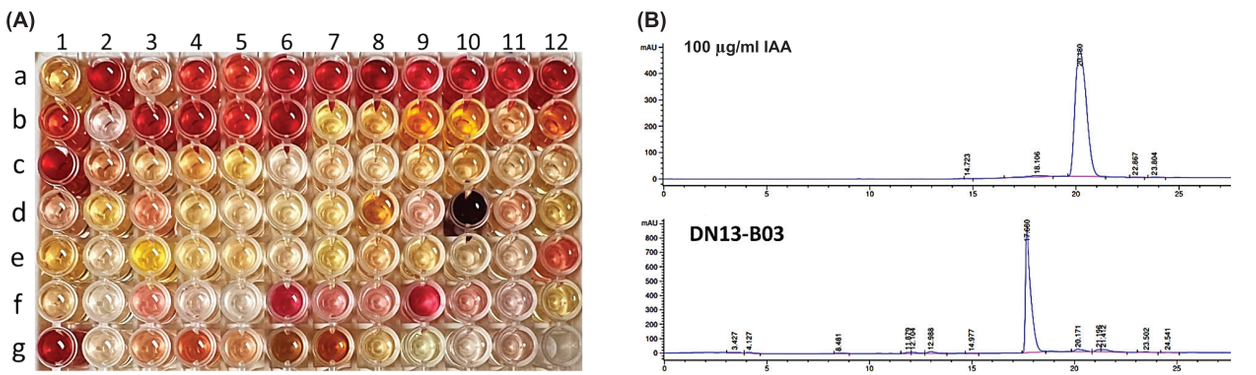

Fig. 1. IAA production activity of isolated microorganism strains. (A) IAA detection in culture broths of isolated microorganism strains using Salkowski reagent. The pink-red coloration indicates positive IAA production, with colour intensity correlating with IAA concentration; wells a1 - a4, b1 - b12, c1 - c12, d1 - d12, e1 - e12, f1 - f12 and g1 - g11: microorganism samples; wells a5 - a8 and a9 - a12: standard IAA samples with concentrations of 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml; wells b9 - b11: negative controls; **(B)** HPLC analysis of IAA production by bacterial strain DN13_B03; (Upper chromatogram) Standard IAA sample at 100 µg/ml; (Lower chromatogram) Culture filtrate of strain DN13_B03. Images are representative of three independent experiments (n=3).

3.2. Identification of microorganism strains with high IAA production

The bacterial strain DN13_B03 exhibited remarkable IAA production capability and was selected for comprehensive characterisation and identification. Morphological examination revealed that strain DN13_B03 formed circular, smooth-edged colonies with a yellowish-white colour when cultured on TSA medium at 28°C for 24 hours (Fig. 2A). The colonies ranged in size from 0.5-2 mm in diameter. Microscopic analysis showed that DN13_B03 cells were Gram-positive, rod-shaped bacteria with dimensions of (0.8-1.5)×(2-5) µm (Fig. 2B). Molecular identification based on *16S rRNA* gene sequence analysis positioned strain DN13_B03 within the genus *Priestia*. Phylogenetic tree analysis (Fig. 2C) revealed that strain DN13_B03 clustered closely with *P. megaterium* DSM 1804, with a bootstrap value of 63%, indicating moderate phylogenetic support.

These morphological characteristics align with the typical features of the genus *Priestia* (formerly *Bacillus*), with the yellowish-white colonies on agar media [19] and rod-shaped, Gram-positive cells observed under microscopic examination being consistent with previous descriptions of *P. megaterium* in the literature [20]. Together, the morphological and molecular analyses provide strong evidence for the taxonomic placement of strain DN13_B03 as *P. megaterium*. It is worth noting that this species was recently reclassified from *Bacillus megaterium* to *P. megaterium* based on comprehensive phylogenomic analyses [21]. The phylogenetic tree also illustrates the relationship between our strain and *Bacillus subtilis* C14-1, highlighting the close evolutionary connection between these genera. The identification of strain DN13_B03 as *P. megaterium* is particularly significant, as this species has previously been reported as a PGPR with the capacity to produce IAA [22]. Several studies have demonstrated that *P. megaterium* can enhance plant growth through multiple mechanisms, including IAA production, phosphate solubilisation, and nitrogen fixation [23].

However, strain DN13_B03 exhibits exceptional characteristics that distinguish it from other previously reported *P. megaterium* strains. Its IAA production level of 178 µg/ml (optimised to 205 µg/ml) is substantially higher than values typically reported for this species. For comparison, S. Wang, et al. (2021) [22] documented that *P. megaterium* WW1211 produced IAA at only 42.5 µg/ml, nearly 5 times lower than strain DN13_B03. This production efficiency surpasses that of widely used commercial PGPR strains such as *Azospirillum brasilense* (50-80 µg/ml IAA) and *Pseudomonas fluorescens* (30-60 µg/ml IAA) [13], positioning our isolate among the most potent natural IAA producers reported to date. This remarkable IAA

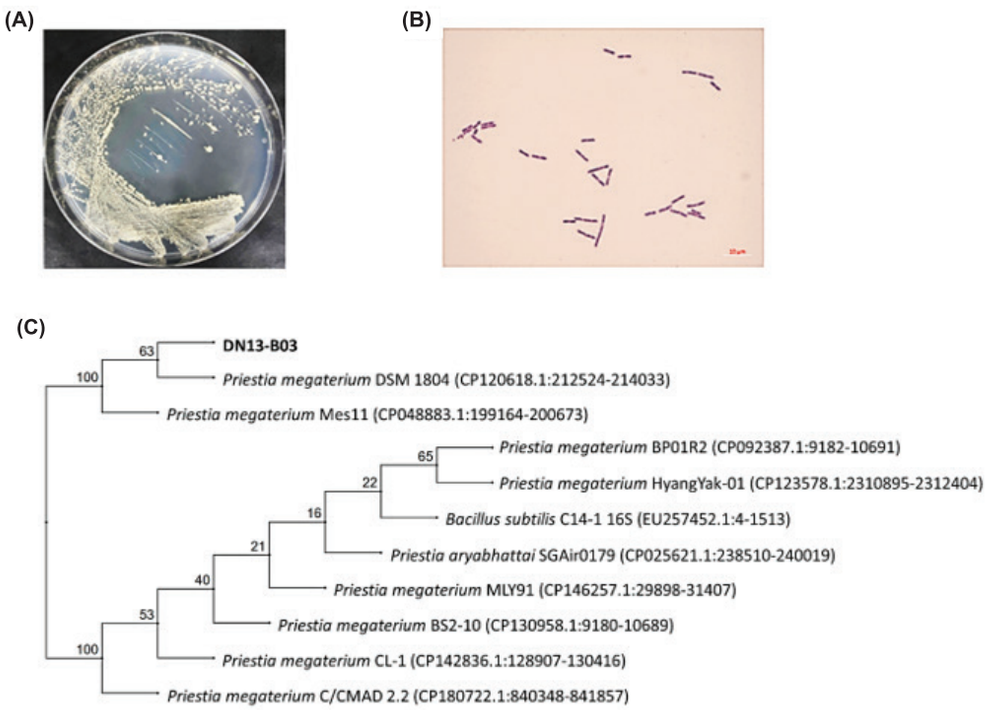

Fig. 2. Morphological and taxonomic characteristics of strain DN13_B03. (A) Colony morphology of bacterial strain DN13_B03 on TSA medium; **(B)** Cell morphology of bacterial strain DN13_B03; **(C)** Phylogenetic tree of strain DN13_B03 constructed based on 16S rRNA gene sequences; bootstrap values are shown as percentages of 1000 replications; accession numbers and reference sequence regions from GenBank are indicated in parentheses.

production capability suggests potential genetic differences between strain DN13_B03 and other *P. megaterium* strains. These differences may include gene duplications in IAA biosynthetic pathways, mutations in regulatory regions enhancing the expression of key enzymes, or the acquisition of novel biosynthetic genes through horizontal gene transfer. Comparative genomic analysis between DN13_B03 and standard *P. megaterium* strains would likely reveal the genetic determinants responsible for its superior IAA production capability.

Another significant aspect of strain DN13_B03's uniqueness lies in its host-specific adaptation to Robusta coffee. Indigenous microorganisms that have co-evolved with specific host plants typically develop specialised adaptations to the particular chemical composition of their host's root exudates [14]. Coffee plants produce a distinctive profile of secondary metabolites, including caffeine and various phenolic compounds, which can be inhibitory to many microorganisms but may act as signalling molecules or carbon sources for specifically adapted strains.

3.3. Effect of environmental factors on IAA production by DN13_B03 strain

To optimise IAA production by the selected strain DN13_B03, various environmental parameters were investigated, including L-tryptophan concentration, temperature, and pH (Fig. 3).

Our investigation of L-tryptophan concentration effects (Fig. 3A) revealed that IAA production was minimal in the absence of L-tryptophan but increased significantly with higher concentrations, reaching approximately 205 µg/ml at 0.5%. This finding indicates that L-tryptophan serves as an essential precursor for IAA biosynthesis in strain DN13_B03, consistent with the tryptophan-dependent pathway reported in many bacteria [24]. The plateau in production observed at concentrations above 1.0% suggests that 0.5-1.0% is the optimal range for industrial or agricultural applications.

Regarding temperature effects (Fig. 3B), strain DN13_B03 exhibited remarkable temperature tolerance, maintaining high IAA production (180-190 µg/ml) across a broad range from 25 to 40°C. This temperature adaptability is particularly advantageous for developing bioinoculants suitable for diverse climatic conditions. Similar temperature optima have been reported for other *Bacillus* species with plant growth-promoting capabilities [25].

The pH study (Fig. 3C) demonstrated that strain DN13_B03 produced maximum IAA (approximately 190 µg/ml) at pH 6.0, with production declining at both higher and lower pH values. This preference for slightly acidic conditions aligns with findings for other IAA-producing bacteria [26] and likely reflects the optimal pH for enzymatic activity in the IAA biosynthetic pathway [27]. The optimal pH of 6.0 for IAA production by strain DN13_B03 represents a significant ecological adaptation to Dak Nong's soil conditions. The Central Highlands region, including Dak Nong province, is characterised by basaltic soils with naturally acidic pH ranging from 4.5 to 6.5. These ferrosols typically become more acidic with age and intensive cultivation. The strain's peak IAA production at pH 6.0 suggests that it has evolved specifically in response to the slightly acidic conditions of Dak Nong's coffee plantations, enhancing its effectiveness as an indigenous biofertiliser. This natural pH alignment eliminates the need for soil amendments when using DN13_B03 as a bioinoculant, providing a sustainable solution that works harmoniously with local soil chemistry while potentially helping buffer against further acidification caused by chemical fertilisers.

Our optimisation results demonstrate that strain DN13_B03 can produce substantial amounts of IAA under specific conditions (0.5% L-tryptophan, temperatures between 25-40°C, and pH 6.0), highlighting its potential as an effective plant growth-promoting inoculant. The strain's broad temperature tolerance, coupled with more specific requirements for L-tryptophan concentration and pH, suggests targeted formulation strategies for agricultural

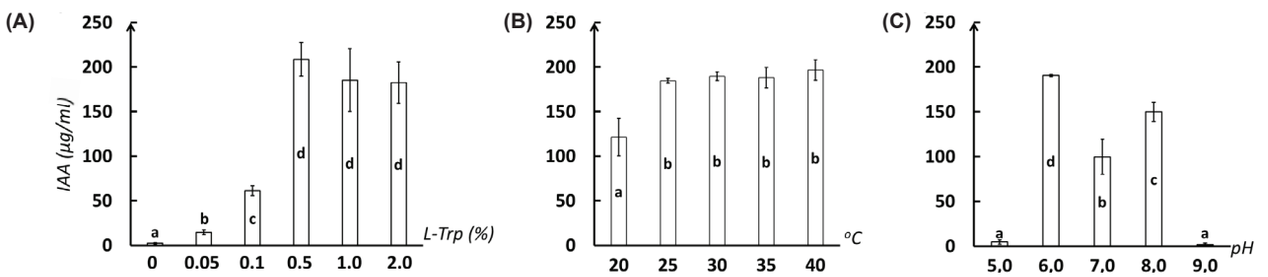

Fig. 3. Effect of culture conditions on IAA production by strain DN13_B03. (A) Bacterial strain DN13_B03 was cultured in medium supplemented with 0.05, 0.1, 0.5, 1.0, and 2.0% L-Tryptophan; **(B)** at temperatures of 20, 25, 30, 35, and 40°C; **(C)** under medium pH conditions of 5.0, 6.0, 7.0, 8.0, and 9.0. Error bars represent standard deviation (SD) of three independent experimental replicates (n=3). Different letters above or inside columns indicate statistically significant differences between mean values (one-way ANOVA followed by Tukey's test, $p<0.05$).

applications. *P. megaterium* strains with comparable IAA production levels have been shown to significantly enhance plant growth parameters in various crops [22, 28].

While the OFAT methodology used in this study provided valuable insights into the effects of individual parameters on IAA production, this approach has several limitations. OFAT cannot detect interactions between variables and may overlook optimal conditions that result from such interactions. Additionally, it requires more experimental runs yet may still fail to identify the true global optimum in complex biological systems. For future optimisation studies, statistical techniques such as response surface methodology (RSM) or other design of experiments (DoE) approaches would be more effective. These methods can evaluate multiple factors simultaneously while requiring fewer experiments overall. RSM, in particular, could generate predictive models describing the relationships between IAA production and various parameters, enabling more precise identification of optimal conditions and factor interactions. Such approaches would be especially valuable for scaling up IAA production for commercial biofertiliser formulations.

It is also important to acknowledge that while the present study provides compelling evidence of high IAA production *in vitro*, it does not include *in planta* validation to directly demonstrate the strain's plant growth-promoting effects. This represents a limitation of the current work, as laboratory IAA production does not always translate directly to proportional plant growth enhancement under field conditions due to complex plant-microbe interactions and environmental factors. Although the high levels of IAA produced by strain DN13_B03 might theoretically promote root development and plant growth in coffee seedlings, this requires empirical confirmation. Future research should therefore prioritise comprehensive plant trials, including seed germination assays, root elongation studies, and seedling growth experiments under both greenhouse and field conditions. These studies should specifically evaluate the effects of DN13_B03 inoculation on coffee root architecture, nutrient uptake efficiency, drought resistance, and overall plant vigour. Additionally, dosage optimisation will be crucial, as excessive IAA concentrations could potentially inhibit rather than promote root growth. We anticipate initiating these validation trials with a particular focus on comparing the performance of DN13_B03 against both untreated controls and commercial biofertiliser products.

4. Conclusions

In conclusion, we identified strain DN13_B03, isolated from the Robusta coffee rhizosphere in Dak Nong Province, as an exceptional IAA producer (178 µg/ml) among 539 microbial isolates. We characterised the strain as *P. megaterium* based on morphological and *16S rRNA* gene sequence analyses. Our optimisation studies demonstrated maximum IAA production (205 µg/ml) at 0.5% L-tryptophan, broad temperature tolerance (25-40°C), and an optimum pH of 6.0. This indigenous strain shows significant potential for developing region-specific biofertilisers to support sustainable coffee cultivation in Vietnam's Central Highlands.

Challenges for practical application include maintaining bacterial viability during formulation and field application and ensuring consistent performance under varying environmental conditions. Future research should focus on field trials in diverse coffee-growing regions, investigating synergistic effects with other beneficial microorganisms, and developing cost-effective formulation technologies to facilitate the commercial deployment of this promising strain.

CRediT author statement

Nguyen Duy Phuong: Conceptualisation, Methodology, Validation, Formal analysis, Data curation, Visualisation, Writing, Reviewing, and Editing; Nguyen Thi Thu Ha: Methodology, Investigation, Formal analysis; Nguyen Thanh Duc: Validation, Writing; Nguyen Le Thanh An: Formal analysis, Editing; Pham Thi Thuy Van, Dam Thi Thanh Ha: Formal analysis, Reviewing and Editing; Nguyen Thanh Ha: Methodology, Investigation, Formal analysis.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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