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UNIVERSITY OF AGRICULTURE AND FORESTRY

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RESEARCH ON NITROGEN-FIXING AND IAA-SYNTHESIZING MICROORGANISMS FOR SELECTED CROPS IN THAI NGUYEN

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SUMMARY OF DOCTORAL DISSERTATION IN BIOTECHNOLOGY

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THE LIST OF PUBLISHED WORKS RELATED TO THE DISSERTATION

1. Van Chi Tran, Pham Thi Tuyet Mai, Nguyen Thi Giang, La Van Hien, Nguyen Manh Tuan, Nguyen Thanh Hai, **Hoang Thi Lan Anh*** and Nguyen Quoc Khuong (2024). Isolation, Selection, and Biological Evaluation for Bacteria that Fix Nitrogen and Produce Indole-3 Acetic Acid from Paddy Soils in Vietnam. The Open Agriculture Journal, pp. 1-16.
2. **Hoang Thi Lan Anh**, Nguyen Thi Giang, Nguyen Manh Tuan, Pham Thi Tuyet Mai, Tran Van Chi (2024). Study on the nitrogen fixation capacity and indole-3-acetic acid (IAA) biosynthesis of *Flavobacterium anhuiense* strain MN47 isolated from tea cultivation soil in Thai Nguyen. Vietnam Journal of Science and Technology, pp. 34–39.
3. Van Chi Tran*, La Van Hien, Nguyen Manh Tuan, Nguyen Thi Giang, **Hoang Thi Lan Anh** (2023). Selection of highly active bacterial strains for nitrogen fixation and indole-3-acetic acid (IAA) synthesis from tea-growing soils in Tuc Tranh commune, Thai Nguyen province. Journal of Forestry Science and Technology, Vol. 12, No. 6, pp. 3–11.
4. **Hoang Thi Lan Anh**, Nguyen Thanh Hai, Nguyen Thi Giang, Pham Thi Tuyet Mai, Nguyen Manh Tuan, Tran Van Chi (2025). Isolation and selection of high-activity nitrogen-fixing and indole-3-acetic acid (IAA) synthesizing bacteria from maize cultivation soils in Thai Nguyen and Ha Giang provinces. Journal of Forestry Science and Technology, Vol. 14, No. 3, pp. 3–11.
5. **Hoang Thi Lan Anh**, Hoang Thi Thuy, Nguyen Thanh Hai, Tran Van Chi (2025). Effects of bio-organic fertilizer containing *Azospirillum* sp. NL1 and *Azotobacter* sp. NL3 on yield and economic efficiency of rice and maize production in Thai Nguyen. Vietnam Journal of Agricultural Science and Technology.

INTRODUCTION

1. Rationale for the Study

Agriculture plays a pivotal role in ensuring food security, serving as a primary source of essential food and agricultural products, significantly contributing to export revenue, and providing employment for the majority of Vietnam's rural population. However, the excessive use of chemical fertilizers - averaging over 10 million tons annually, which is three times the global average - with inorganic fertilizers accounting for approximately 75% of total consumption, has led to significant consequences. These include soil degradation, environmental pollution, and potential risks to public health (Vu Van Thuc, 2024).

In response to this situation, bio-organic fertilizers (HCVS) are considered a strategic solution for restoring soil fertility, improving nutrient use efficiency, and reducing the reliance on chemical fertilizers (Lukas Schütz et al., 2017). To promote organic agriculture, the Government of Vietnam has issued several supportive policies, including Decision No. 885/QĐ-TTg (2020), Decision No. 5190/QĐ-BNN-BVTV (2023), and notably Resolution No. 36-NQ/TW (2023) on the development of biotechnology. Among these efforts, the application of nitrogen-fixing microorganisms - which enable crops to absorb atmospheric nitrogen - along with the microbial synthesis of indole-3-acetic acid (IAA) to stimulate plant growth, is receiving increasing attention in both research and practical implementation.

Thai Nguyen province, known for its agricultural strengths in key crops such as tea, rice, and maize, is also facing increasing environmental pollution caused by the excessive use of chemical fertilizers. Therefore, the dissertation topic titled **"Study on nitrogen-fixing and IAA-producing microorganisms for selected crops in Thai Nguyen"** holds significant practical relevance. It aims to provide an environmentally friendly biological solution to improve crop productivity and promote sustainable agriculture.

This dissertation forms part of the research outcomes of the national-level genetic resources science and technology project entitled ***"Exploration and development of microbial genetic resources capable of nitrogen fixation and indole-3-acetic acid (IAA) biosynthesis for***

application in agricultural production”, project code: NVQG-2021/ĐT.04

2. Research Objective

To isolate, select, and identify microbial strains capable of nitrogen fixation and indole-3-acetic acid (IAA) biosynthesis for the production of bio-organic fertilizer applicable to selected crops in Thai Nguyen province.

3. Scientific and practical significance

a. Scientific Significance

This dissertation is a comprehensive scientific study on the biological characteristics of selected nitrogen-fixing and indole-3-acetic acid (IAA)-producing microbial strains. The findings contribute to the development of bio-organic fertilizers that enhance crop productivity in Thai Nguyen province. The research provides valuable scientific material for further studies, teaching, and practical applications in the production of bio-organic fertilizers.

b. Practical Significance

The isolation and selection of nitrogen-fixing and IAA-producing microbial strains for application in the production of bio-organic fertilizers contribute to increasing crop yields, reducing dependence on chemical fertilizers, and improving economic outcomes for farmers. This represents an important step toward developing advanced cultivation models that align with the trends of green, safe, and sustainable agriculture, while also protecting public health.

4. Novel Contributions of the Dissertation

- Successfully isolated, selected, and identified microbial strains with high capacities for nitrogen fixation and indole-3-acetic acid (IAA) biosynthesis through biological characterization, 16S rRNA gene sequencing, and whole-genome sequencing.

- Determined the optimal application rates of bio-organic fertilizer containing selected strains of Azospirillum sp. and Azotobacter sp. for rice and maize in Thai Nguyen province. Specifically, an application rate of 1.5 tons/ha for rice resulted in a 16.62% yield increase, while 2.0 tons/ha for maize led to a 15.67% yield increase.

- Assessed the economic efficiency of using bio-organic fertilizer on rice and maize crops in Thai Nguyen. The results showed that the bio-organic fertilizer model increased economic returns by 10,062,000 VND (equivalent to 35.7%) for rice and 11,044,000 VND (equivalent to 39.6%) for maize compared to the control model.

- Opened a new direction for research and application in agricultural production by addressing the challenge of reducing nitrogen input in crop cultivation. The study also demonstrated the potential of microorganisms to produce the plant growth regulator indole-3-acetic acid (IAA), thereby enhancing crop growth performance and yield.

Chapter 1. LITERATURE REVIEW

1.1. The Role of Microorganisms in Agricultural Ecosystems

1.1.1. Microorganisms and the Decomposition of Organic Matter

1.1.2. Plant Growth-Promoting Bacteria (PGPB)

1.1.3. Microorganisms and Biological Control of Pests and Diseases

1.1.4. Microorganisms and Soil Health Improvement

1.1.5. Microorganisms and Agricultural Sustainability

1.2. Overview of Plant Growth-Promoting Microorganisms

1.2.1. Definition of Plant Growth-Promoting Microorganisms

1.2.2. Characteristics of Plant Growth-Promoting Microorganisms

1.3. Nitrogen-Fixing and IAA-Producing Microorganisms

1.3.1. Microorganisms Capable of Nitrogen Fixation

1.3.2. Microorganisms Capable of Indole-3-Acetic Acid (IAA) Biosynthesis

1.3.3. Microorganisms with Both Nitrogen-Fixing and IAA-Producing Capabilities

1.3.4. Factors Affecting Nitrogen Fixation and IAA Biosynthesis

1.3.5. Application of Genetic Analysis in Microbial Strain Selection

1.4. Overview of Bio-Organic Fertilizers

1.5. Research Status in Vietnam and Worldwide on Microorganisms with Dual Capabilities of Nitrogen Fixation and IAA Biosynthesis Applied in Bio-Organic Fertilizer Production

1.5.1. International Studies

1.5.2. Studies in Vietnam

1.6. Current Status of Maize and Rice Cultivation and the Application of Biotechnology in Agricultural Production in Thai Nguyen Province

1.7. Key Findings from the Literature Review

Chapter 2. OBJECTIVES, SCOPE, CONTENT, AND RESEARCH METHODS

2.1. Objectives, scope, location, and duration

a. Research objectives

- Several microbial strains capable of nitrogen fixation and indole-3-acetic acid (IAA) biosynthesis were isolated from various sources, including soils cultivated with rice, tomato, maize, peanut, and tea; as well as from peanut and soybean roots. Samples were collected from 10 provinces and cities: Thai Nguyen, Tuyen Quang, Yen Bai, Vinh Phuc, Cao Bang, Ha Giang, Hanoi, Bac Kan, Lang Son, and Bac Giang.

- The SUMO rice variety, a pure-line cultivar commonly grown in Thai Nguyen, has a growth duration of 105–110 days, provided by Vietnam Agricultural Development and Investment Consulting Joint Stock Company.

- The genetically modified single-cross hybrid maize variety NK4300 (F1) is widely cultivated in Thai Nguyen province, provided by Vietnam Agricultural Development and Investment Consulting Joint Stock Company.

b. Scope of the Study

- Spatial scope: Microbial strains capable of nitrogen fixation and indole-3-acetic acid (IAA) biosynthesis were isolated, selected, and identified from various sources, including cultivated soils and plant roots collected in Hanoi and several northern midland and mountainous provinces. Physiological, biochemical, and biological experiments were conducted at the Fermentation Technology Laboratory and the Molecular Biology Laboratory of the University of Agriculture and Forestry – Thai Nguyen University. Field trials were carried out in Phu Luong and Phu Binh districts, Thai Nguyen province.

- Temporal scope: Secondary data collection, experimental research, and evaluations of effectiveness were conducted during the period from 2021 to 2024.

2.2. Research Content

- Isolation and selection of microbial strains capable of nitrogen fixation and indole-3-acetic acid (IAA) biosynthesis
- Study of the biological characteristics of selected microbial strains
- Production of bio-organic fertilizer from selected microbial strains
- Evaluation of the effectiveness of bio-organic fertilizer containing selected microbial strains on rice and maize cultivation (0.5 ha per model).

2.3. Research Methods

2.3.1. Methods for Isolation and Selection of Nitrogen-Fixing and IAA-Producing Microorganisms

- *Collection of secondary data*
- *Isolation and selection of microbial strains capable of nitrogen fixation and indole-3-acetic acid (IAA) biosynthesis*
- *Analysis of quality indicators*
- *Study of cell morphology, colony characteristics, and Gram staining properties*
- *Method for total DNA extraction from bacterial cells*
- *Species-level identification of microbial strains based on 16S rRNA gene sequencing*
- *Whole-genome sequencing of selected microorganisms*
- *Evaluation of microbial interaction potential among selected strains*

2.3.2. Methods for Evaluating the Biological Characteristics of Selected Strains

- *Assessment of the physiological characteristics of the selected microbial strains*
- *Optimization of culture conditions (incubation time, temperature, pH) for selected strains*
- *Evaluation of the biochemical characteristics of the selected strains*
- *Analysis of the genetic characteristics of the selected strains*
- *Biosafety evaluation of the two selected microbial strains*

2.3.3. Methods for Producing Bio-Organic Fertilizer from Selected Microbial Strains

- Production of microbial inoculants
- Production of bio-organic fertilizer:

- Method for studying the effects of bio-organic fertilizer application rates on rice and maize

a. Study on bio-organic fertilizer application rates for rice cultivation

b. Study on bio-organic fertilizer application rates for maize cultivation

2.3.4. Methods for Evaluating the Effectiveness of Bio-Organic Fertilizer Containing Selected Microbial Strains on Rice and Maize (Model Scale: 0.5 ha per Trial)

- Evaluation of crop yield in rice and maize cultivation models using bio-organic fertilizer

- Evaluation of the economic efficiency of rice and maize production models using bio-organic fertilizer

2.3.5. Data Analysis Methods

Data were compiled, analyzed, and optimized using the following software programs: SAS 9.1, Design-Expert (DX 7.1.5), JASP 0.19.3, and Microsoft Excel 2015.

CHAPTER 3. RESEARCH RESULTS AND DISCUSSION

3.1. Results of Isolation and Selection of Microbial Strains Capable of Nitrogen Fixation and IAA Biosynthesis

3.1.1. Isolation Results of Microbial Strains Capable of Nitrogen Fixation and IAA Biosynthesis

From 175 collected samples of soil and plant roots, 80 nitrogen-fixing microbial strains were isolated using the selective Ashby nitrogen-free medium. These strains were subsequently screened for IAA synthesis through the Salkowski colorimetric assay under culture conditions supplemented with L-tryptophan. The results showed that 38 strains (47.5%) exhibited both biological activities—nitrogen fixation and IAA synthesis—mainly derived from soil samples associated with tea (39.47%), maize (31.58%), and rice (14.42%).

3.1.2. Screening of Potential Strains for Nitrogen Fixation and IAA Biosynthesis Activity

Quantitative evaluation of nitrogen fixation and IAA synthesis revealed that 14 out of 38 isolated strains were capable of synthesizing

IAA at concentrations ≥ 50 µg/ml, and 6 out of 38 strains demonstrated nitrogen fixation capacity ≥ 20 µg/ml (NH_4^+), including NL1, NL2, MN88, MN42, MN47, and MN72. These six strains, which exhibited both high nitrogen fixation ability (≥ 50 µg/ml) and IAA production (≥ 20 µg/ml), were classified as potential candidates and selected for further study. These results are consistent with previous findings by Nguyen Anh Huy and Nguyen Huu Hiep (2018), Liu et al. (2019), Myo et al. (2019), and Rodrigues et al. (2016) regarding nitrogen-fixing and IAA-producing bacteria.

3.1.3. Morphological Characteristics of Screened Microbial Strains

Among the six screened strains, five were identified as Gram-negative, while only one strain (MN88) was Gram-positive. The cell morphology was primarily in two forms: short rods and ovoid-shaped cells. All six strains exhibited motility.

3.1.4. Identification of Microbial Strains Based on 16S rRNA Gene Sequencing

Table 3.4. Comparison of 16S rRNA gene sequence similarity of the six selected microbial strains with the closest species published in the EzBioCloud (EzTaxon) database.

No.	Strain Code	Closest Species	Similarity (%)
1	NL1	*Azospirillum lipoferum* NCIMB 11861^T (Z29619)	99,36
2	NL3	*Azotobacter vinelandii* IAM 15004^T (AB175657)	100,00
3	MN88	*Bacillus tropicus* $N24^T$ (MACG01000025)	99,20
4	MN72	*Agrobacterium deltaense* YIC 4121^T (MRDI01000025)	99,85
5	MN47	*Flavobacterium anhuiense* $D3^T$ (EU046269)	99,86
6	MN42	*Stenotrophomonas pavanii* DSM 25135^T (LDJN01000038)	99,93

The 16S rRNA gene sequence analysis of strain NL1 revealed the highest similarity (99.36%) to *Azospirillum lipoferum* NCIMB 11861^T (accession number: Z29619). Strain NL3 showed 100% similarity to *Azotobacter vinelandii* IAM 15004^T (AB175657). Strain MN88 exhibited 99.20% similarity to *Bacillus tropicus* N24^T (MACG01000025). Strain MN72 was 99.85% similar to *Agrobacterium deltaense* YIC 4121^T (MRDI01000025). Strain MN47 showed the highest similarity (99.86%) to *Flavobacterium anhuiense* D3^T (EU046269), while strain MN42 demonstrated 99.93% similarity to *Stenotrophomonas pavanii* DSM 25135^T (LDJN01000038).

Comparative analysis of 16S rRNA gene sequences using the EzBioCloud database and phylogenetic tree construction confirmed the identification of the bacterial strains as *Azospirillum lipoferum* NL1, *Azotobacter vinelandii* NL3, *Bacillus tropicus* MN88, *Agrobacterium deltaense* MN72, *Flavobacterium anhuiense* MN47, and *Stenotrophomonas pavanii* MN42. These genera represent promising candidates for the development of microbial bioinoculants and biofertilizers with nitrogen-fixing and indole-3-acetic acid (IAA)-producing capacities that support plant growth and contribute to improved crop yield and quality (Cruz-Hernández et al., 2022; Aasfar et al., 2021; Ortiz et al., 2024; Kaur et al., 2022; Seo et al., 2024).

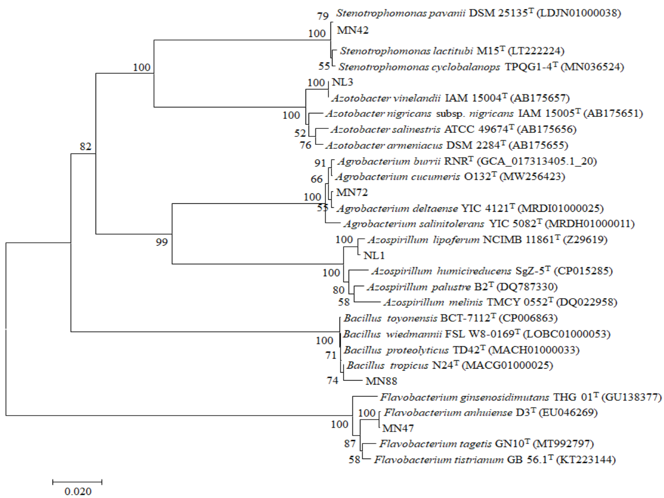

Figure 3.2. Phylogenetic tree of six selected microbial strains

3.1.5. Study on the Interaction Capabilities Among Screened Strains

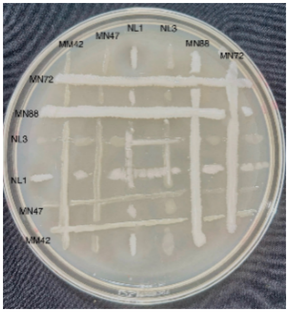

Figure 3.3. Interaction capabilities among the screened microbial strains

Based on the results presented in Figure 3.3, strains NL1 and NL3 exhibited the highest nitrogen fixation and IAA synthesis activities, and showed no signs of mutual inhibition during co-cultivation. Therefore, NL1 and NL3 were selected for further studies.

3.2. Results of the Study on the Biological Characteristics of Selected Strains

3.2.1. Evaluation of the Physiological Characteristics of the Selected Microbial Strains

3.2.1.1. Growth Characteristics of the Selected Microbial Strains

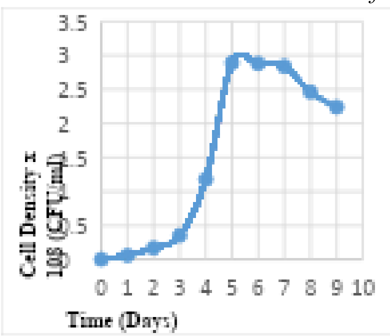

Figure 3.4A. Growth Curve of Strain NL1

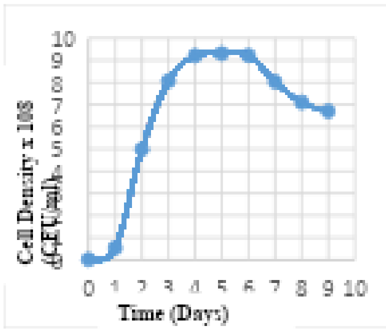

Figure 3.4 B. Growth Curve of Strain NL3

The results shown in Figures 3.4A and 3.4B indicate that the two selected microbial strains reached their highest cell densities at different levels under identical culture conditions (Ashby medium), with both achieving maximum density after 5 days of incubation. Specifically, *Azotobacter sp.* NL3 reached a peak cell density of 9.307×10^8 CFU/ml, while *Azospirillum sp.* NL1 achieved a maximum of 2.892×10^8

CFU/ml after 5 days of cultivation.

3.2.1.2. Effects of Temperature and pH on the Growth of Selected Microbial Strains

a. Effect of Temperature

Table 3.5. Effect of Incubation Temperature on the Growth of Two Selected Microbial Strains

No.	Strain Code	Cell density (×10⁸ CFU/mL) after 5 days of incubation at corresponding temperatures							
		28°C	30°C	32°C	34°C	36°C	38°C	40°C	42°C
1	*Azospirillum* sp. NL1	2,882	2,892	**2,942**	2,802	1,362	0,972	0,142	0,002
2	*Azotobacter* sp. NL3	9,067	9,307	**9,337**	8,087	6,127	1,197	0,507	0,007

The results indicated that temperature had a significant effect on the growth of the two selected microbial strains. *Azospirillum sp.* NL1 and *Azotobacter sp.* NL3 exhibited good growth within the temperature range of 28–32°C, with 32°C identified as the optimal temperature. At temperatures exceeding 34°C, the growth of both strains was strongly inhibited. These findings are consistent with previous studies by Nguyen Thi Minh (2017), Nguyen Tien Dung (2011), and Nguyen Thi Hang Nga (2016), which also demonstrated that the suitable temperature range for *Azospirillum sp.* and *Azotobacter sp.* lies between 28°C and 32°C. Beyond this threshold, cell proliferation and biomass accumulation decreased markedly.

b. Effect of Culture Medium pH on Growth

Table 3.6. Effect of pH on the Growth of Two Selected Microbial Strains

No.	Medium pH	Cell density (×10⁸ CFU/mL) after 5 days of incubation at the corresponding medium pH levels	
		Azospirillum sp. NL1	*Azotobacter* sp. NL3
1	pH = 4,0	-	-
2	pH = 4,5	0,012	0,007
3	pH = 5,0	1,052	0,757
4	pH = 5,5	1,942	2,087
5	pH = 6,0	2,742	8,957
6	pH = 6,5	**3,002**	9,297
7	pH = 7,0	2,942	**9,337**

8	pH = 7,5	2,852	9,197
9	pH = 8,0	2,272	7,117
10	pH = 8,5	1,092	1,617
11	pH = 9,0	0,022	0,097

The results in Table 3.6 show that *Azotobacter sp.* NL3 achieved the highest cell density (9.337 × 10^8 CFU/mL) at pH 7.0, whereas *Azospirillum sp.* NL1 reached 3.002 × 10^8 CFU/mL at pH 6.5.

These findings are consistent with previous studies by Nguyen Thi Hang Nga (2016), Nguyen Thi Minh (2017), and Nguyen Tien Dung (2011), all of which reported that *Azotobacter* and *Azospirillum* strains exhibit optimal growth within the pH range of 6.5–7.0.

3.2.2. Optimization of Cultivation Conditions (Incubation Time, Temperature, and pH) for Selected Strains

The desirability function method was applied to optimize cell density during cultivation using Design-Expert software (DX 7.1.5). A total of 43 experimental runs were generated for each strain, and the optimal condition for maximizing the response variable (cell density) was predicted. For strain NL1, the optimal predicted conditions were: Incubation time: 5.22 days; Temperature: 31.73 °C; pH: 6.61

Under these conditions, the predicted cell density was 3.12159 × 10^8 CFU/ml. For strain NL3, the optimal predicted conditions were: Incubation time: 5.44 days; Temperature: 32.18 °C; pH: 7.10

Under these conditions, the predicted cell density was 9.77929 × 10^8 CFU/ml. These results were found to be highly consistent with experimental validation, as presented in Figures 3.4 and 3.5.

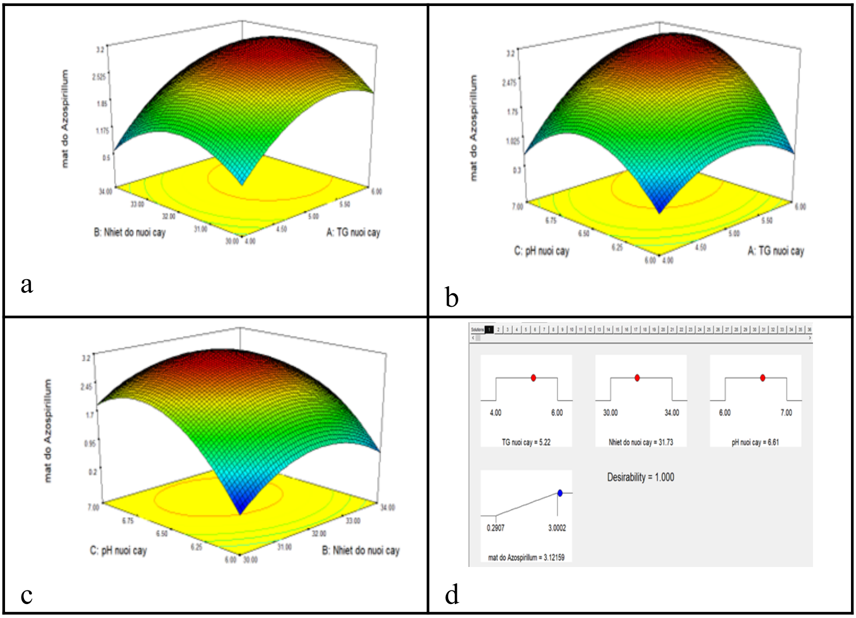

Figure 3.5. Response surface of cell density for strain NL1

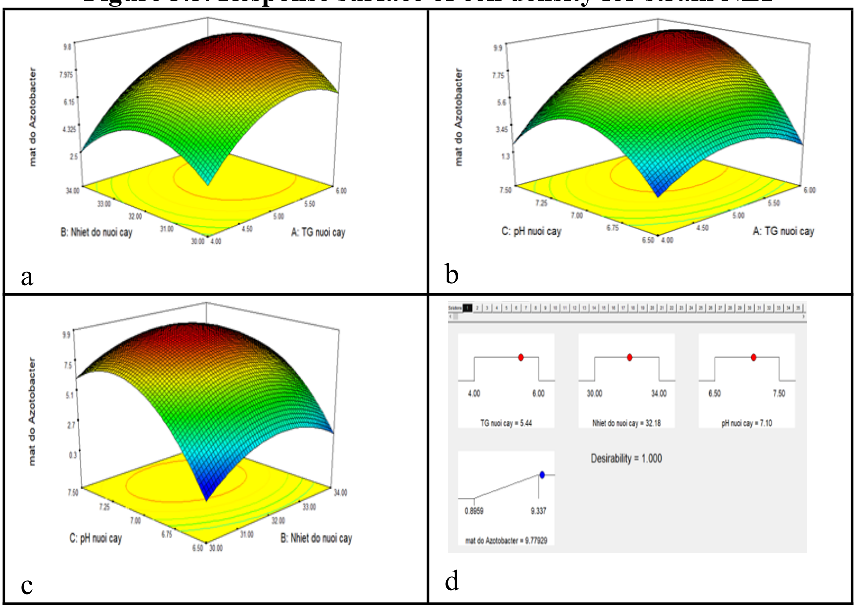

Figure 3.6. Response surface of cell density for strain NL3

3.2.3. Evaluation of the Biochemical Characteristics of the Selected Microbial Strains

3.2.3.1. Enzymatic Activity of the Selected Microbial Strains

The qualitative assessment of enzymatic activity in the two selected microbial strains revealed the following: out of the 19 enzymes tested, 7 enzymes showed positive reactions in both strains, while 6 enzymes were not expressed in either strain. The strain exhibiting the highest number of enzyme activities was *Azospirillum* sp. NL1, with 11 out of 19 enzymes testing positive using the diagnostic kit. Meanwhile, *Azotobacter* sp. NL3 displayed enzymatic activity for 9 out of 19 enzymes tested.

3.2.3.2. Fermentation and Assimilation of Sugars, Indole Production, Catalase and Oxidase Activities, Methyl Red and Voges–Proskauer Reactions, KIA and TSI Tests of the Selected Strains.

Based on the biochemical reactions and test results using API kits (20NE and 32GN), the abilities of the selected strains - such as indole production, catalase and oxidase activities, methyl red reaction, Voges-Proskauer (VP) test, KIA and TSI reactions, carbon source utilization, and nitrate reduction to nitrite - were assessed. Among the 53 biochemical indicators examined, 20 were expressed by both strains, while 11 indicators were negative in both strains. The number of positive biochemical traits differed between the strains: *Azospirillum sp.* NL1 showed 37 positive traits out of 53, whereas *Azotobacter sp.* NL3 displayed 25 positive traits.

3.2.4. Genetic Characterization of the Selected Strains

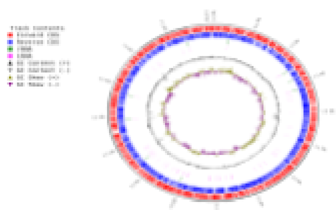

Figure 3.7A. Genome map of *Azospirillum* sp. strain NL1

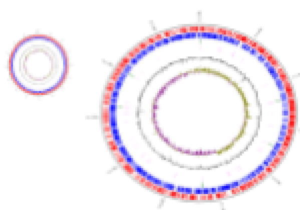

Figure 3.7 B. Genome map of Azotobacter sp. strain NL3

Based on the genome analysis results, the presence of genes related to the nitrogen fixation pathway was confirmed in the genome sequences of the studied strains. Specifically, the genome of *Azospirillum* sp. NL1 contains 29 genes involved in nitrogen fixation and 4 genes associated with indole-3-acetic acid (IAA) biosynthesis. In contrast, the genome of *Azotobacter* sp. NL3 was found to harbor 45 genes involved in nitrogen fixation and 7 genes associated with the IAA biosynthetic pathway, based on annotations from the MetaCyc database.

3.2.5. Classification and Biosafety Evaluation of the Two Selected Microbial Strains

The 16S rRNA gene sequences of strains NL1 and NL3 have been deposited in the GenBank database under accession numbers OR125575 and OR125577, respectively. Strain NL1 showed 99.36% sequence similarity to the type strain *Azospirillum lipoferum* 11861T (Z29619) based on EzBioCloud analysis. Whole-genome DNA-DNA hybridization (DDH) between NL1 and the reference strain yielded a similarity of 99.67%, which exceeds the species delineation threshold (≥70%) according to the Genome-to-Genome Distance Calculator (GGDC) v3.0. Therefore, strain NL1 was identified as *Azospirillum lipoferum* NL1 and classified as Biosafety Level 1 (BSL-1) according to the American Type Culture Collection (ATCC) guidelines.

Table 3.12. Genome Hybridization Results of Strains NL1 and Azotobacter sp. NL3 with Closely Related Species via Genome-to-Genome Distance Calculator (GGDC) v3.0

Closest type strain	**Genome hybridization value (DDH, %)**	
	Azospirillum sp. NL1 (PRJNA1031402)	*Azotobacter* sp. NL3 (PRJNA1031787)
Azospirillum lipoferum NCIMB 11861^T (VTTN000000000)	99,67	-
Azotobacter vinelandii IAM 15004^T (BSFG01000000)	-	99,54

Note: "–", indicates a different genus; DDH: DNA-DNA Hybridization.

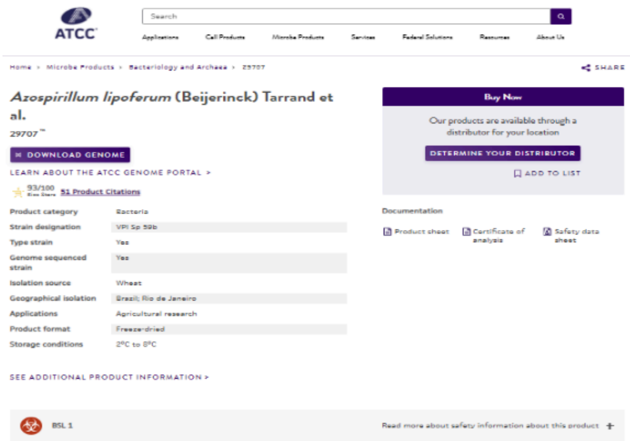

Figure 3.8. Reference strain data of *Azospirillum lipoferum* published by ATCC (USA)

Similarly, the strain *Azotobacter* sp. NL3 is classified as a biosafety level 1 microorganism (Figure 3.9). The 16S rRNA gene sequence of *Azotobacter* sp. NL3 (length: 1,531 bp; GenBank accession number: OR125577) showed 100% similarity to the reference strain *Azotobacter vinelandii* IAM 15004T (AB175657). Whole-genome sequence comparison between strain NL3 (CP137764) and *Azotobacter vinelandii* IAM 15004T (BSFG01000000) revealed a 99.54% genome-to-genome similarity (Table 3.12). Based on these results, strain *Azotobacter* sp. NL3 is taxonomically classified under the genus *Azotobacter*, and designated as *Azotobacter vinelandii* NL3 (OR125577).

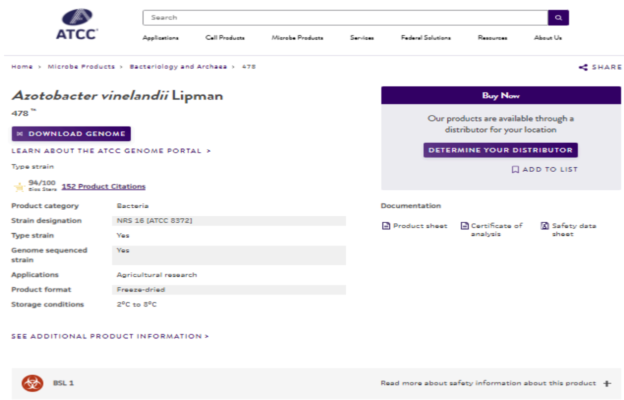

Figure 3.9. Reference data of *Azotobacter vinelandii* species published by ATCC (USA)

3.3. Production of Bio-Organic Fertilizer from Selected Microbial Strains

3.3.1. Production of Microbial Inoculants

Applying the microbial inoculant production protocol coded MREC-QTCN-2022-02, the study conducted three consecutive production batches for each inoculant, with a capacity of 50 kg per batch. A total of 300 kg was produced, including Inoculant 1 containing *Azospirillum* sp. NL1 (CPNL1), and Inoculant 2 containing *Azotobacter* sp. NL3. The quality parameters of the inoculants after production are presented in Table 3.13.

Table 3.13. Quality Parameters of Microbial Inoculants Produced by the Protocol MREC-QTCN-2022-02

No.	Type of inoculant containing microbial strain	Density (CFU/g)	Contaminating microorganisms (CFU/g)	Moisture content (%)	Biological activity	
					Nitrogen-fixing activity (µg/ml)	IAA-producing activity (µg/ml)
1	*Azospirillum* sp. NL1	$6{,}89 \times 10^{10}$	Not detected	$9{,}4^{a}$	$22{,}16^{b}$	$109{,}38^{b}$
2	*Azotobacter* sp. NL3	$9{,}69 \times 10^{10}$	Not detected	$9{,}5^{a}$	$24{,}55^{a}$	$119{,}13^{a}$

Note: *In the same column, values with different superscript letters are significantly different at $P < 0.05$.*

The results presented in Table 3.13 indicate that the analytical parameters of the produced microbial inoculants meet the required standards for the production of bio-organic fertilizers, in accordance with Circular No. 09/2019/TT-BNNPTNT dated August 27, 2019, issued by the Ministry of Agriculture and Rural Development (currently the Ministry of Agriculture and Environment).

3.3.2. Production of Bio-organic Fertilizer

Applying the production protocol for bio-organic fertilizer coded MREC-QTCN-2022-03, the project conducted five consecutive production batches and obtained a total of 2,000 kg of bio-organic fertilizer.

Table 3.14. Quality Parameters of Bio-organic Fertilizer from Three Production Batches

No.	Indicators	Test Method	Batch 1	Batch 2	Batch 3	Standard (TCVN)	Evaluation
1	Required maturity (humification)	TCVN 7185:2002	Good	Good	Good	Good	Passed
2	Particle size uniformity	TCVN 7185:2002	Uniform	Uniform	Uniform	Uniform	Passed
3	Moisture content (%)	TCVN 9297:2012	25.33	25.38	25.34	≤ 35	Passed
4	pH	TCVN 5979:2007	6.7	6.5	6.6	6.0 – 8.0	Passed
5	Microbial cell density	TCVN 6166:2002, 10784:2015				$\geq 10^6$ CFU/g	Passed
5.1	Azospirillum sp. NL1 ($\times 10^8$ CFU/g)		2.21	2.23	2.19		
5.2	Azotobacter sp. NL3 ($\times 10^8$ CFU/g)		4.15	4.16	4.14		
6	Total organic matter (%)	TCVN 9294:2012	36.15	36.19	36.20	≥ 22	Passed
7	Total nitrogen content (%)	TCVN 8557:2010	2.72	2.69	2.72	≥ 2.5	Passed
8	Available phosphorus (% P_2O_5)	TCVN 8559:2010	3.67	3.70	3.70	≥ 2.5	Passed
9	Available potassium (% K_2O)	TCVN 8560:2018	1.77	1.79	1.78	≥ 1.5	Passed
10	Salmonella spp. (CFU/25g)	TCVN 10780-1:2017	Not Detected	Not Detected	Not Detected	0	Passed
11	Lead content (mg/kg)	TCVN 9290:2012	118.12	118.16	118.17	≤ 200	Passed
12	Cadmium content (mg/kg)	TCVN 9291:2012	1.19	1.24	1.26	≤ 2.5	Passed
13	Chromium content (mg/kg)	TCVN 10674:2015	97.73	97.79	97.82	≤ 200	Passed

14	Nickel content (mg/kg)	TCVN 10675:2015	38.61	38.57	38.56	≤ 100	Passed
15	Mercury content (mg/kg)	TCVN 10676:2015	0.19	0.17	0.18	≤ 2	Passed

The results in Table 3.14 show that the quality of the bio-organic fertilizer produced across the three batches was consistent. All sensory, quality, and safety indicators of the bio-organic fertilizer met the requirements set forth in the Vietnamese National Standard TCVN 7185:2002 on microbial organic fertilizers.

3.3.3. Study on the Effect of Bio-organic Fertilizer Dosage on Rice and Maize Crops

3.3.3.1. Results of Studying the Application Rate of Bio-organic Fertilizer on Rice

The results presented in Tables 3.15 and 3.16 show that the actual yields (NSTT) of treatments CT2, CT3, and CT4 in both the spring and summer seasons were significantly higher than those of CT1 and the control. There were no statistically significant differences in yield among CT2, CT3, and CT4 ($P \geq 0.05$). Therefore, considering both the level of investment and the yield achieved in both seasons, the application of 1.5 tons/ha of bio-organic fertilizer (treatment CT2) was the most effective and was selected for model expansion and economic efficiency evaluation in subsequent experiments.

Table 3.15. Effects of Bio-Organic Fertilizer Application Rates on Rice Yield in the Spring Season

No.	Treatment	Bio-organic Fertilizer Dose (tons/ha)	Effective Panicles/m²	Grains/Spike	Filled Grains/Spike	Filled Grain Rate (%)	1000-grain Weight (g)	Theoretical Yield (quintals/ha)	Actual Yield (quintals/ha)
1	ĐC	0	$235{,}13^{b}$	$158{,}00^{e}$	$122{,}50^{a}$	77,53	23,50	67,69	$54{,}12^{c}$
2	CT1	1,0	$246{,}60^{ab}$	$165{,}30^{d}$	$124{,}10^{a}$	75,08	24,12	73,81	$59{,}05^{b}$
3	**CT2**	**1,5**	**$250{,}60^{a}$**	**$167{,}37^{c}$**	**$130{,}00^{a}$**	**77,67**	**24,48**	**79,75**	**$63{,}81^{a}$**
4	CT3	2,0	$251{,}60^{a}$	$169{,}00^{b}$	$130{,}00^{a}$	76,92	24,49	80,10	$64{,}07^{a}$
5	CT4	2,5	$251{,}80^{a}$	$170{,}30^{a}$	$130{,}00^{a}$	76,34	24,50	80,20	$64{,}13^{a}$

CV (%)	2,86	0,3	3,81	-	-	-	3,73
$LSD_{0,05}$	13,32	0,96	9,14	-	-	-	3,28

Note: In the same column, mean values with different letters are significantly different at P < 0,05

Table 3.16. Effect of bio-organic fertilizer application rates on rice in the summer–autumn crop

No.	Treatment	Application Rate of Bio-Organic Fertilizer (tons/ha)	Effective Panicles/m²	Grains/ Panicle	Filled Grains/ Panicle	Filled Grain Rate (%)	1000-grain Weight (g)	Theoretical Yield (quintals/ha)	Actual Yield (quintals/ha)
1	ĐC	0	233,13[b]	163,93[a]	106,87[b]	65.19	23,10	57,55	46,03[c]
2	CT1	1,0	244,67[ab]	165,40[a]	110,00[b]	66.51	23,80	64,05	51,23[b]
3	**CT2**	**1,5**	**250,40[a]**	**167,70[a]**	**119,00[a]**	**70.96**	**24,00**	**71,51**	**57,19[a]**
4	CT3	2,0	250,60[a]	169,00[a]	119,13[a]	70.49	24,00	71,65	57,30[a]
5	CT4	2,5	250,67[a]	170,53[a]	119,40[a]	70.02	24,00	71,83	57,44[a]
CV (%)			3,26	3,67	3,15	-	-	-	4,91
$LSD_{0,05}$			15,08	11,56	6,81	-	-	-	4,98

Note: In the same column, mean values with different letters are significantly different at P < 0,05

3.3.3.2. Results of the Study on the Application Rate of Bio-organic Fertilizer on Maize

Table 3.17. Effect of Bio-organic Fertilizer Application Rates on Maize in the Spring Crop

No.	Treatment	Application Rate of Bio-Organic Fertilizer (tons/ha)	No. of Effective Ears/Plant (ears)	No. of Rows/Ear (rows)	No. of Kernels/Row (kernels)	Weight of 1000 Kernels (g)	Theoretical Yield (quintals/ha)	Actual Yield (quintals/ha)
1	ĐC	0	0,93[a]	12,5[c]	31,63[a]	357,03	78,77	55,12[c]
2	CT1	1,0	0,96[a]	12,6[b]	31,86[a]	376,03	86,95	60,86[b]
3	CT2	1,5	0,97[a]	12,6[b]	33,27[a]	377,03	91,99	62,43[b]
4	CT3	2,0	**0,98[a]**	**12,6[b]**	**33,13[a]**	**389,77**	95,67	**66,94[a]**
5	CT4	2,5	0,98[a]	12,7[a]	33,23[a]	390,03	96,79	67,74[a]
CV (%)			3,74	9,11	9,98	-	-	3,08
$LSD_{0,05}$			0,07	22	6,13	-	-	3,63

Note: In the same column, mean values with different letters are significantly different at P < 0,05

Table 3.18. Effect of Bio-Organic Fertilizer Dosage on Maize in the Summer-Autumn Crop

No.	Treatment	Application Rate of Bio-Organic	No. of Effective Ears/Plant (ears)	No. of Rows/Ear (rows)	No. of Kernels/Row (kernels)	Weight of 1000 Kernels (g)	Theoretical Yield (quintals/ha)	Actual Yield (quintals/ha)

		Fertilizer (tons/ha)						
1	Control	0	0,92[a]	12,5[c]	30,51[d]	348,62	73,39	51,39[c]
2	CT1	1,0	0,94[a]	12,6[b]	31,9[c]	354,06	80,26	56,20[b]
3	CT2	1,5	0,95[a]	12,6[b]	32,33[b]	354,06	82,21	57,56[ab]
4	CT3	2,0	**0,98[a]**	**12,7[a]**	**32,82[a]**	**361,86**	88,69	**61,46[a]**
5	CT4	2,5	0,97[a]	12,7[a]	33,06[a]	361,78	88,40	61,90[a]
CV (%)			3,46	0	0,44	-	-	4,07
$LSD_{0,05}$			0,06	0	0,27	-	-	4,42

Note: In the same column, mean values with different letters are significantly different at P < 0,05

The results in Tables 3.17 and 3.18 show that all treatments using bio-organic fertilizer in both spring and summer maize crops produced significantly higher yields compared to the control, with statistically significant differences at P<0,05. The actual yields of treatments CT3 and CT4 were the highest; however, there was no statistically significant difference in actual yield between CT3 and CT4 (P≥0,05). Therefore, considering the level of investment and the yield obtained in both spring and summer seasons, the application of bio-organic fertilizer at 2,0 tons/ha (Treatment CT3) was the most effective and should be selected for scaling up the model and evaluating economic efficiency in subsequent experiments.

3.4. Evaluation of the effectiveness of bio-organic fertilizer containing selected microbial strains on rice and maize (at a scale of 0,5 ha per model)

3.4.1. Evaluation of crop yield from the application model of bio-organic fertilizer on rice and maize

3.4.1.1. Rice yield in the model using bio-organic fertilizer

The results presented in Table 3.19 indicate that the actual yield from the field model using the bio-organic fertilizer (AZO-NL) was comparable to the model using commercial bio-organic fertilizer, with yields of 6.323 tons/ha and 6.325 tons/ha, respectively. The difference between these two treatments was not statistically significant (P ≥ 0.05).

Both models, however, achieved significantly higher yields compared to the control model that used only composted manure. Specifically, the model using the AZO-NL bio-organic fertilizer resulted in a 16.62% increase in actual yield compared to the model without bio-organic fertilizer application. Our findings are consistent with those reported by Nguyễn Anh Huy and Nguyễn Hữu Hiệp (2018), who observed a 10–30% increase in rice yield when applying bio-organic fertilizer under field conditions. Similarly, Rajasekar et al. (2022) demonstrated that the use of nitrogen-fixing microorganisms such as Azospirillum and Azotobacter in combination with organic fertilizers increased rice yield by 15–20% compared to traditional cultivation methods.

Table 3.19. Yield of the Field Model Using Bio-organic Fertilizer on Rice

Indicator / Treatment	Number of panicles/m² (panicles)	Number of grains/panicle (grains)	Number of filled grains/panicle (grains)	Filled grain ratio (%)	1000-grain weight (g)	Growth duration (days)	Theoretical yield (quintals/ha)	Actual yield (quintals/ha)
Formula 1 (CT1)	236,00^{b}	163,13^{a}	122,20^{b}	74,91	23,50^{a}	115,00	67,77	54,22^{b}
Formula 2 (CT2)	251,20^{a}	168,00^{a}	129^{a}	76,79	24,40^{a}	115,00	79,07	63,25^{a}
Formula 3 (AZO-NL)	250,20^{a}	168,00^{a}	130,00^{a}	77,38	24,30^{a}	115,00	79,04	63,23^{a}
CV (%)	2,2	2,1	2,2	-	5,0	-	-	5,6
$LSD_{0,05}$	12,2	8,0	6,5	-	2,8	-	-	7,7

Note: *Within the same column, mean values with different letters are significantly different at P < 0,05. CT1: application of composted manure; CT2: application of commercial bio-organic fertilizer; CT3: application of AZO-NL bio-organic fertilizer.*

3.4.1.2. Yield of the model using bio-organic fertilizer on maize

Table 3.20. Yield of the model using bio-organic fertilizer on maize

Indicator / Treatment	Plant Density (plants/ha)	Ears per Plant (ear)	Kernel Rows per Ear (rows)	Kernels per Row (kernels)	Kernels per Ear (kernels)	1000-Kernel Weight (g)	Potential Yield (quintals/ha)	Actual Yield (quintals/ha)
Formula 1 (CT1)	60.000	1	14	32^{b}	448^{b}	358,5^{b}	96,37	77,10^{b}
Formula 2 (CT 2)	60.000	1	14	35^{a}	478^{a}	390,3^{a}	114,78	89,20^{a}
Formula 3 (AZO-NL)	60.000	1	14	34^{ab}	470,3^{ab}	390,4^{a}	111,48	89,18^{a}
CV (%)	-	-	-	3,0	2,5	1,5	-	2,9
$LSD_{0,05}$	-	-	-	2,3	26,4	13	-	5,7

Note: *Within the same column, mean values with different letters are significantly different at P*

< 0,05. CT1: application of composted manure; CT2: application of commercial bio-organic fertilizer; CT3: application of AZO-NL bio-organic fertilizer.

The results presented in Table 3.20 show that the actual yields of the maize field model using bio-organic fertilizer (AZO-NL) and the model using commercial bio-organic fertilizer were statistically similar, with yields of 89,18 and 89,20 quintals/ha, respectively ($P \geq 0,05$). However, both models had significantly higher yields compared to the control model using composted farmyard manure. The actual yield of the field using AZO-NL bio-organic fertilizer increased by 15,67% compared to the field without bio-organic fertilizer and was equivalent to the yield obtained using commercial bio-organic fertilizer. These findings are consistent with those of Patel et al. (2022) in India, who reported that the application of bio-organic fertilizers increased maize yields by up to 18%. Similarly, Müller et al. (2021) found that the use of microbial-based organic fertilizers improved maize yields by 15–25%.

3.4.2. Evaluation of the Economic Efficiency of the Bio-Organic Fertilizer Application Model on Rice and Maize

3.4.2.1. Evaluation of the Economic Efficiency of the Bio-Organic Fertilizer Application Model on Rice

Table 3.21. Results of Economic Efficiency Evaluation of the Bio-Organic Fertilizer Application Model on Rice

Unit: VND

No.	Cost Items	Unit	Formula 1 (Đ/C 1)			Formula 2 (Đ/C 2)			Formula 3		
			Quantity	Unit Price	Amount	Quantity	Unit Price	Amount	Quantity	Unit Price	Amount
I	**Total cost**		**46.952.000**			**49.202.000**			**47.702.000**		
1	Seeds	Kg	35	35.000	1.225.000	35	35.000	1.225.000	35	35.000	1.225.000
2	Urea (N)	Kg	271	15.000	4.065.000	271	15.000	4.065.000	271	15.000	4.065.000
3	Phosphorus (P)	Kg	360	6.000	2.160.000	360	6.000	2.160.000	360	6.000	2.160.000
4	Potassium (K)	Kg	116	22.000	2.552.000	116	22.000	2.552.000	116	22.000	2.552.000
5	Traditional organic fertilizer	Kg	8.000	1.000	8.000.000	-	-	-	-	-	-
6	Bio-organic fertilizer	Kg	-	-	-	1.500	5.000	7.500.000	1.500	4.000	6.000.000
7	Chemical pesticides	Pack	125	20.000	2.500.000	65	20.000	1.300.000	65	20.000	1.300.000
8	Herbicide applicatio	Number of times	2	400.000	800.000						
9	Puddling labor					2	400.000	800.000	2	400.000	800.000
10	Labor		171	150.000	25.650.000	200	150.000	30.000.000	200	150.000	30.000.000
II	**Total revenue**	VND/ha	**65.064.000**			**75.900.000**			**75.876.000**		
	Actual yield	quintal/ha	54,22	12.000	65.064.000	63,25	12.000	75.900.000	63,23	12.000	75.876.000
III	**Profit (II-I)**	VND/ha	**18.112.000**			**26.698.000**			**28.174.000**		
IV	Yield increase of Formula 3 over Control 1	%	**16,62**								
V	Profit diff. F3 and F1	VND/ha	**10.062.000 (35,7)**								

No.	Cost Items	Unit	Formula 1 (Đ/C 1)			Formula 2 (Đ/C 2)			Formula 3		
			Quantity	Unit Price	Amount	Quantity	Unit Price	Amount	Quantity	Unit Price	Amount
		(%)									
VI	Profit diff. F3 and F2	VND/ha	1.476.000								

Unit prices are based on Decision No. 726/QĐ-BNN-KN dated February 24, 2022, issued by the Ministry of Agriculture and Rural Development on the promulgation of national-level technical and economic norms for agricultural extension. Formula 1: application of decomposed manure; Formula 2: application of commercial bio-organic fertilizer; Formula 3: application of AZO-NL bio-organic fertilizer

Based on the results of the model implementation, rice yield in the field using AZO-NL bio-organic fertilizer was 16,62% higher than that of Control 1, and the profit was 1,56 times greater. This improvement is attributed to the application of the bio-organic fertilizer at the optimal rate of 1,500 kg/ha, which optimized nutrient availability, leading to better growth of Sumo rice plants, an increase in the number of panicles, and more filled grains per panicle. As a result, the actual yield reached 63,23 quintal/ha, demonstrating higher efficiency.

If considering only the cost of fertilizers, the model field using bio-organic fertilizer (AZO-NL) incurred 2.000.000 VND/ha less than Control Field 1 and 1.500.000 VND/ha less than Control Field 2. This cost reduction is due to the use of bio-organic fertilizer at an optimal dosage of 1.500 kg/ha without the need for additional organic manure, which significantly reduced fertilizer expenses. Furthermore, commercial bio-organic fertilizers are approximately 1.000 VND/kg more expensive than the AZO-NL product.

3.4.2.2. Evaluation of the economic efficiency of the model using bio-organic fertilizer on maize

Table 3.22. Economic efficiency evaluation results of the model using bio-organic fertilizer on maize

Unit: VND

No.	Cost Items	Unit	Formula 1 (Đ/C 1)			Formula 2 (Đ/C 2)			Formula 3		
			Quantity	Unit Price	Amount	Quantity	Unit Price	Amount	Quantity	Unit Price	Amount
I	Seeds		44.925.000			45.525.000			43.525.000		
1	Urea (N)	Kg	45	45000	2.025.000	45	45000	2.025.000	45	45000	2.025.000
2	Phosphorus (P)	Kg	410	15000	6.150.000	410	15000	6.150.000	410	15000	6.150.000
3	Potassium (K)	Kg	480	6000	2.880.000	480	6000	2.880.000	480	6000	2.880.000
4	Traditional organic fertilizer	Kg	135	22000	2.970.000	135	22000	2.970.000	135	22000	2.970.000
5	Bio-organic fertilizer	Kg	8.000	1000	8.000.000	-	-	-	-	-	-
6	Chemical pesticides	Kg	-	-	-	2000	5000	10.000.000	2000	4000	8.000.000
7	Seeds	Pack	125	20.000	2.500.000	85	20000	1.700.000	85	20000	1.700.000

No.	Cost Items	Unit	Formula 1 (Đ/C 1)			Formula 2 (Đ/C 2)			Formula 3		
			Quantity	Unit Price	Amount	Quantity	Unit Price	Amount	Quantity	Unit Price	Amount
8	Labor		136	150000	20.400.000	132	150000	19.800.000	132	150000	19.800.000
II	**Total revenue**	VND/ha	**61.680.000**			**71.360.000**			**71.344.000**		
	Actual yield	quintal/ha	77,10	8.000	61.680.000	89,20	8.000	71.360.000	89,18	8.000	71.344.000
III	**Profit (II-I)**	VND/ha	**16.775.000**			**25.8350.000**			**27.819.000**		
IV	Yield increase of Formula 3 over Control 1	%	**15,67**								
V	Profit diff. F3 and F1	VND/ha (%)	**11.044.000 (39,70)**								
VI	Profit diff. F3 and F2	VND/ha	**1.984.000 (7,13%)**								

Unit prices are based on Decision No. 726/QĐ-BNN-KN dated February 24, 2022, issued by the Ministry of Agriculture and Rural Development on the promulgation of national-level technical and economic norms for agricultural extension. Formula 1: application of decomposed manure; Formula 2: application of commercial bio-organic fertilizer; Formula 3: application of AZO-NL bio-organic fertilizer

Based on the model implementation results, the maize yield in the field using AZO-NL bio-organic fertilizer was 15,67% higher than that of the control field using only composted manure. The profit generated was 1,66 times higher compared to the control. Moreover, when considering fertilizer costs alone, both fields incurred the same expense (8 million VND/ha). This is also an important factor in encouraging farmers to become more aware of converting manure into bio-organic fertilizer, which not only reduces input costs but also improves crop yields and economic efficiency. Additionally, it contributes to protecting the ecological environment and safeguarding human health.

CONCLUSION AND RECOMMENDATIONS

1. Conclusion

- From 175 soil and root samples collected across 10 locations, 80 nitrogen-fixing microbial strains were isolated using the selective nitrogen-free Ashby medium. Among these, 38 strains exhibited both nitrogen fixation and IAA synthesis capabilities. Six strains with the highest bioactivity—NL1, NL3, MN88, MN42, MN47, and MN72—demonstrated nitrogen fixation levels ≥ 20 µg/ml (NH_4^+) and IAA production ≥ 50 µg/ml, and all showed motility.

- The results of 16S rRNA gene sequencing revealed that the selected strains shared 99.2–100% similarity with known species published in GenBank and EzBioCloud databases. Among them, two strains, NL1 and NL3, showed no antagonistic interactions and were selected for the development of a bioinoculant.

- When cultured on Ashby medium, strain NL1 reached its highest cell density after 5 days of incubation, while strain NL3 peaked after 4 days. The optimal temperature for both strains was 32°C. The optimal pH for NL1 and NL3 was 6,5 and 7,0, respectively. These results were consistent with the outcomes of culture optimization experiments designed using the Box-Behnken model.

+ *Azospirillum* sp. strain NL1: The optimal temperature and pH for biomass production in liquid culture were 31.73 °C and 6.61, respectively. This strain exhibited 11 out of 19 enzyme activities and tested positive for 37 out of 53 biochemical indicators.

+ *Azotobacter* sp. strain NL3: The optimal temperature and pH for biomass production in liquid culture were 32.18 °C and 7.10, respectively. This strain exhibited 9 out of 19 enzyme activities and tested positive for 25 out of 53 biochemical indicators.

- Genome analysis results showed that strain NL1 possessed 29 nitrogen fixation genes and 4 IAA biosynthesis genes; strain NL3 had 45 nitrogen fixation genes and 7 genes involved in IAA metabolic pathways, based on MetaCyc database annotations. Both selected microbial strains, NL1 and NL3, were classified as Biosafety Level 1, meeting the safety requirements for bio-organic fertilizer production.

- Produced 300 kg of microbial inoculants with cell density $\geq 10^{10}$ CFU/g, including two types: CPNL1 containing strain NL1 and CPNL3 containing strain NL3 (150 kg each).

- Successfully produced 2.000 kg of bio-organic fertilizer meeting the national quality standard QCVN 01-189:2019/BNNPTNT on fertilizer quality and the TCVN 7185:2002 standard on bio-organic fertilizers.

- Determined optimal application rates for the bio-organic fertilizer: 1.5 tons/ha for rice and 2.0 tons/ha for maize.

- Actual yield evaluation on field models using the bio-organic fertilizer showed yields of 6.32 tons/ha for rice and 8.92 tons/ha for maize.

- Economic efficiency analysis revealed that the rice model achieved a 16,62% increase in yield and a profit increase of 10.062.000 VND (equivalent to 35,7%) compared to the control; the maize model achieved a 15,67% increase in yield and a profit increase of 11.044.000 VND (equivalent to 39,6%) compared to the control.

2. Recommendations

- Continue to study the expression of functional genes related to nitrogen fixation and IAA biosynthesis under different environmental conditions;
- Continue monitoring the effects of bio-organic fertilizers on soil health, and evaluate product quality over multiple growing seasons;
- Expand trials of bio-organic fertilizers on other crops across different areas of Thai Nguyen province;
- Regulatory agencies should develop policies to encourage farmers to shift toward organic and green agriculture practices, thereby promoting and expanding the market for organic and bio-organic fertilizers.