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Nitrogen-fixing and IAA-producing bacterial strains isolated from maize soil in Vietnamese mountainous regions

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Abstract: Nitrogen (N) is the most limiting factor for crop yield, especially in acidic soils. A biological approach should be taken as the usage of chemical N fertilizer is expensive and harmful to both human health and the environment. Therefore, the current study aimed at isolating a promising N_2-fixing bacteria (NFB) that can provide IAA as an indicator of a plant growth promoter. The origin of isolation was in the mountainous areas in the North of Vietnam. The finest isolate according to IAA and N-fixing. Then, the biochemical and genomic traits of the selected isolate were determined. Eight of the twelve NFB strains identified in the study were able to produce IAA. The IAA-producing NFB showed an amount of 18.882–109.381 NH_4^+ and 4.321–22.158 IAA (µg/ml). The NL1 strain was chosen because of its exceptional performance compared to the other strains. The morphology and biochemical processes of the NL1 strain were determined. Its growth condition was as follows: duration (A) of 5.22 days, temperature (B) of 31.73°C, and pH (C) of 6.61 at the maximum bacteria density of 3.12159×10^8 CFU/ml. After that, the NL1 strain was identified as *Azospirillum* sp. and contained 12 N_2-fixing genes and 4 IAA-producing genes. This indicates the potential of the *Azospirillum* sp. NL1 strain as an N_2 fixer and an IAA producer. Thus, to increase soil fertility and crop productivity, and partially replace chemical fertilizer for sustainable agriculture, it should be further investigated in greenhouse and field studies.

Keywords: *Azospirillum*, bacterial genome, biochemistry, indole acetic acid, mountainous regions, nitrogen-fixing bacteria

Introduction

Nitrogen (N) use efficiency is extremely important to agriculture and the environment (Govindasamy et al., 2023). N is an irreplaceable element for plant growth and yield (Shehu, 2025). To improve crop yield, farmers often use chemical N fertilizers containing urea [$CO(NH_2)_2$] (Chen et al., 2024). Given the high demand for food from the world's expanding population, chemical N fertilizer use is unavoidable and growing ever more essential (Santos et al., 2021). However, when N fertilizer leaks into the soil and water, the NUE can be lowered by 50% (Yadav et al., 2023), which is called nitrate pollution, affecting human and soil health (Craswell, 2021). Furthermore, N fertilizer may contribute to greenhouse gas emissions, which would contribute to climate change (Aryal et al., 2022; Filonchyk et al., 2024). Therefore, the overuse of N fertilizers causes pollution in soil, underground water, we well as air, food contamination, and climate change (Ali, 2025). Nevertheless, using less chemical N fertilizer reduces crop yield and lowers farmers' income (Van Wesenbeeck et al., 2021), while using it too much causes environmental problems (Tyagi et al., 2022), especially in regions featuring poor soil fertility (Naorem et al., 2023). Vietnam's Thai Nguyen province is a typical instance, where the soil is classified as Kanhaplustult ultisoils (Huu Chien et al., 2018; Minh and Anderson, 2021), i.e. the soil has low pH and low nutrient content, including N. Additionally, in an acidic environment, the availability of nutrients such as N, phosphorus (P), calcium, potassium (K), and

magnesium decreases while the toxicities of Al, Fe, and Mn increase (Shetty et al., 2021; Wang X et al., 2023). Furthermore, long-term N fertilization can only marginally increase the soil's overall N content, which can negatively impact its overall P and K levels in addition to causing environmental issues (Nguyen et al., 2001).

To address these issues, there have been many approaches, such as slow-release N fertilizer (Priya et al., 2024), a combination of N fertilizer with organic materials (Wan et al., 2021), and N_2-fixing bacteria (NFB) (Shalaby et al., 2023). Of these approaches, the usage of NFB is regarded to be more sustainable and eco-friendly (Alomari et al., 2024). The principle of N_2 fixation is the reduction of free N_2 molecules in the atmosphere to NH_4^+ under microaerobic conditions by the facilitation of suitable enzymes, also called nitrogenases (Rodelas, 2021). These nitrogenases have three types, including Mo-, V-, and Fe- nitrogenase (Chanderban et al., 2023). These N-fixing enzymes are reported to be found in some plants, especially the legume (Wang Q et al., 2023), which has been reported to intercrop with rice (Wang S et al., 2022), maize (Sahoo et al. 2023), and wheat (Liu et al., 2023), i.e. these plants can fix N_2. Their symbiotic connection with the rhizospheric and endophytic NFB is the main reason for this (Aziz et al., 2022). Some well-known NFB are rhizobia, cyanobacteria, and Frankia (Sepp et al., 2023). Furthermore, by increasing plants' resistance to stress, these bacterial species can encourage plant development as well (Aasfar et al., 2021) and synthesize plant growth-promoting substances (PGPS), e.g., indole acetic acid (IAA) (Isti'anah et al., 2021), gibberellins (Nett et al., 2022), and cytokinin (Singh et al., 2024). Among these PGPS, IAA is well-known for promoting plant height and root length (Gao et al., 2022) and can be used as an indicator to evaluate the plant growth-promoting capacity of a bacterial strain (Van Dinh et al., 2024). It is vital to find an NFB candidate to partially replace chemical N fertilizer; therefore, IAA and NH4+ productions are useful criteria to screen for better strains (Dabban et al., 2024).

To enhance soil health and plant productivity for sustainable agriculture, an indigenous NFB strain should be chosen and described in light of the challenges with NUE worldwide, and specifically in Thai Nguyen, Vietnam. Thus, the current study was conducted to determine good NFB strains in Thai Nguyen soils, identify the best strain based on its production of IAA and NH4+, and then analyze the identified strain based on its morphological, biochemical, and genomic characteristics.

Results

Nitrogen fixing bacteria (NFB) isolation

Table 1 demonstrates that 12 NFB strains have been isolated from Thai Nguyen province's seven communes. Among the NFB, eight strains can produce IAA. Table 2 shows the results of further testing on these strains to determine their production of IAA and NH_4^+. The NH_4^+ production ranged from 24.61 μg/ml, while the IAA production was recorded as 11.33-119.13 μg/ml. Among them, the greatest amount of NH_4^+ and IAA belonged to the NL1 strain. Therefore, this strain was chosen for the following experiment. This agrees with previous studies, where some NFB strains were reported to produce IAA (Isti'anah et al., 2021; Gang et al., 2021, Ouyang et al., 2022). For example, in the study by Zhang X et al. (2022), an NFB strain of *Curtobacterium* sp. A02 isolated from cassava roots can produce 13.38 mg L^{-1} of N and 1.56 mg L^{-1} of IAA, which results in plant growth improvement. However, in the study by Uzma et al. (2022), the results were 0.18 and 116 mg L^{-1}, respectively, by the *Pseudomonas aeruginosa* strain. As can be seen, there are differences among NH_4^+ and IAA productions of different NFB. Therefore, the isolation and selection of newly indigenous NFB are necessary to fully exploit the potential of these NFB.

Table 1. Results of the isolation of bacteria capable of nitrogen fixation and IAA synthesis

STT	Strain symbol	Sampling location	Nitrogen fixation capacity	IAA Aggregation Capabilities
1	**NL1**	On Luong Commune	+	+
2	NL33	On Luong Commune	+	-
3	NL35	Co Lung Commune	+	+
4	NL38	Co Lung Commune	+	+
5	NL39	Dong Dat Commune	+	-
6	NL321	Hop Thanh Commune	+	+
7	NL335	Hop Thanh Commune	+	+
8	NL336	Phan Me Commune	+	-
9	NL338	Phan Me Commune	+	+
10	NL324	Yen Ninh Commune	+	+
11	NL331	Yen Ninh Commune	+	-
12	NL341	Yen Trach Commune	+	+

Table 2. Comparison of nitrogen fixation and IAA synthesis of 12 isolated strains

No.	Strain label	Nitrogen fixation capacity (µg/ml)	IAA synthesis capacity (µg/ml)
1	**NL1**	**22.158**	**109.381**
2	NL33	3.213	-
3	NL35	11.465	68.981
4	NL38	7.865	35.421
5	NL39	2.146	-
6	NL321	15.843	72.684
7	NL335	9.472	26.347
8	NL336	2.163	-
9	NL338	12.615	56.782
10	NL324	4.321	18.882
11	NL331	3.113	-
12	NL341	8.918	23.112

Besides NH_4^+ and IAA, these NFB can also function as P solubilizer (Maulida et al., 2024), K solubilizer (Devi et al., 2022), and siderophore producer (Zhang Y et al., 2025). Therefore, these functions should be further investigated.

NFB morphological description

Among the above 12 NFB strains, 8 strains with greater NH_4^+ and IAA productions were chosen for the morphological description, including NL1, NL35, NL38, NL321, NL335, NL338, NL324, and NL341. All eight strains had an oval, shiny, and slimy surface and were white or almost white in color overall (Fig. S2, S3; Table S1). This is consistent with previous studies describing the morphology of NFB, which is Gram-negative with white colonies (Liang et al., 2023; Seidu et al., 2025). Especially for the NL1 strain, its morphology is consistent with that of the *Azospirillum* spp. described by Ogunniyi et al. (2023) and Ali (2024). The NL1 strain was further investigated for biochemical traits based on the NH_4^+ and IAA productions. Fig. 1 displays the strain NL1's growth curve.

Culture time (days)	UV Absorption	Bacterial density x 10^8 (CFU/ml)
0	0.007	0.002
1	0.013	0.062
2	0.024	0.172
3	0.042	0.352
4	0.125	1.182
5	**0.296**	**2.892**
6	0.295	2.882
7	0.291	2.842
8	0.253	2.462
9	0.231	2.242

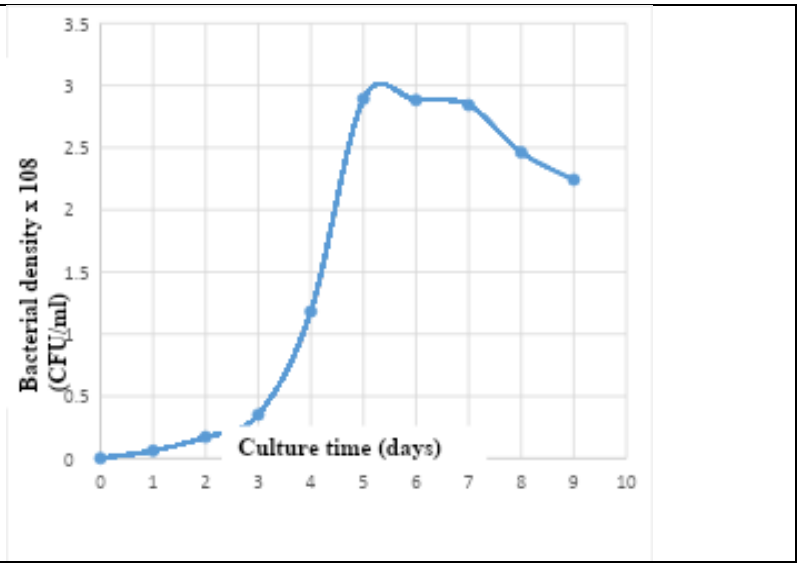

Fig 1. Growth curve of strain NL1.

NL1 strain growth according to pH and temperature

Effects of temperature and pH on NL1 strain growth

The growth of the NL1 strain ranged from 0.002 to 2.942 x 10^8 CFU/ml and from 0.012 to 3.002 x 10^8 CFU/ml according to the temperature and pH factors, respectively (Tables 3 and 4). The ideal temperature and pH were 32°C and 6.5, respectively. This is consistent with the optimal conditions of *Azospirillum* spp. described in the study by Romero-Perdomo et al. (2015), in which the optimal conditions were roughly 32°C in temperature and 6.8 in pH.

Growth function of the NL1 strain according to Box-Behnken modal.

Table 5 illustrates that three factors were evaluated: culture duration (A), temperature (B), and pH (C). The bacterial density equation was found as follows: Y = + 2.94 + 0.62*A + 0.023*B + 0.58*C + 0.12*A*B + 0.47*A*C – 0.15*B*C – 0.76*A2 – 0.84*B2 – 1.1*C2. While the temperature and pH conditions reduced the bacterial density, the time in conjunction with either temperature or pH indicated a positive bacterial density.

Table 3. Effect of culture temperature on strains.

Strain symbol	Cell density (x 10^8 CFU/ml) after 5 days of culture at temperature Corresponding Survey							
	28°C	30°C	32°C	34°C	36°C	38°C	40°C	42°C
NL1	2.882	2.892	**2.942**	2.802	1.362	0.972	0.142	0.002

Table 4. Effect of environmental pH on strain growth.

Strain symbol	Cell density (x 10^8 CFU/ml) after 5 days of culture at the pH of corresponding survey environment					
	pH = 4.0	pH = 4.5	pH = 5.0	pH = 5.5	pH = 6.0	pH = 6.5
NL1	-	0.012	1.052	1.942	2.742	**3.002**
	pH = 7.0	pH = 7.5	pH = 8.0	pH = 8.5	pH = 9.0	
	2.942	2.852	2.272	1.092	*0.022*	

The data in Table 6 were analyzed to test the model's significance and compatibility. ANOVA analysis showed that the probability value of the model P-value < 0.0001 < 0.05. Therefore, the selected model was significant with a regression coefficient R^2 = 0.9965. This outcome demonstrates that 99.65% of the experimental data agrees with the model's expected data. 43 options were identified when the density of NL1 cells acquired from the culture procedure was optimized using the expectation function method utilizing Design-Expert software (DX 7.1.5). The best option predicted to maximize the objective function was: culture time 5.22 days, temperature 31.73 °C, pH = 6.61 (Fig S4). Then the NL1 cell density achieved under the above conditions was calculated to be 3.12159 x 10^8 CFU/ mL (Fig. S5). This result is highly compatible with the experimental test results, presented in Fig. S5.

Table 5. Three-factor Box-Behnken experimental matrix and NL1 cell density under different culture conditions.

No.	Transformation			*Azospirillum* Cell Density (10^8CFU/ml)
	A – incubation time	B- Culture temperature	C-pH Culture	
1	4.00	30.00	6.50	0.7093
2	6.00	30.00	6.50	1.8141
3	4.00	34.00	6.50	0.6396
4	6.00	34.00	6.50	2.2211
5	4.00	32.00	6.00	0.4186
6	6.00	32.00	6.00	0.6279
7	4.00	32.00	7.00	0.5931
8	6.00	32.00	7.00	2.6862
9	5.00	30.00	6.00	0.2907
10	5.00	34.00	6.00	0.5117
11	5.00	30.00	7.00	1.7908
12	5.00	34.00	7.00	1.4187
13	5.00	32.00	6.50	2.9072
14	5.00	32.00	6.50	3.0002
15	5.00	32.00	6.50	3.0002
16	5.00	32.00	6.50	2.9072
17	5.00	32.00	6.50	2.8839

Notes: A: Culture time (days); B: Culture temperature (°C); C: Culture pH.

Table 6. ANOVA variance analysis results of the NL1 strain culture model.

Source	Standard F	P Value
Model	222.61	<0.0001
Lack of Fit	5.53	0.066
R2	0.9965	

Note: Standard F: Fisher Standard; "Lack of Fit": A standard for evaluating the incompatibility of a model with experiments.

Enzyme production by the NL1 strain

Table S2 shows that the NL1 strain can produce 11 of the 20 enzymes examined in the current study. The enzymes produced were alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, ß-glucuronidase, and N-acetyl-ß-glucosaminidase. It can be concluded that the NF3 strain can conduct protein, carbohydrate, and lipid hydrolysis and dephosphorylation (Cuatlayotl-Olarte et al., 2023). Table S3 further illustrates the NL1 strain's biochemical characteristics.

Results of genome analysis, nitrogen fixation-related functional gene analysis, and IAA synthesis of the NL1 strain

Genomic sequencing of the NL1 strain

The raw sequences were purified to exclude adapters, low-quality nucleotides, and duplicate reads to provide a full genome sequence of strain NL1 with excellent quality for further investigations. Table S4 demonstrates the quality results before and after purification. The total number of sequences before and after purification showed that the Illumina sequencing data had a %Q30 index > 80%, meeting the requirements to start the *de novo* assembly step.

Table S5 demonstrates the assembly results, showing that the total length of the contigs is 8,071,173, consistent with the *Azospirillum* genome size of 7-8 Mb. The largest contig has a length of 2,813,755 bp, accounting for 34.86% of the total length of the contigs; the G+C% ratio is 67.23%. Detailed results are shown in the analysis result files shown in Table 5. The numbers of coding sequences (CDS), tRNAs, and rRNAs of strain NL1 are 7313, 79, and 26, respectively. *Azospirillum* species are plant growth-supporting bacteria belonging to the alpha subclass of Proteobacteria. The report of Martin-Didonet et al (2000) determined that the genome size of *Azospirillum* species, including *A. brasilense, A. lipoferum, A. amazonense, A. irakense, A. halopraeferens,* and *A. largimobile,* ranged from 4.8 to 9.7 Mbp. Thus, the results of this study are consistent with the genome size of the genus *Azospirillum*.

The genome map of strain NL1 was also constructed (Fig. 2). The analysis results show that from the center outward, circle 1 illustrates the GC slope. Circle 2 shows the GC content (peaks outside/inside the circle indicate values higher or lower than the average G+C content, respectively). Circle 3 represents the ncRNA genes. Circles 4, 5, and 6 represent the CDSs, with colors corresponding to the COG, KEGG, and GO categories, respectively. Circle 7 shows the protein coding sequence with predicted function.

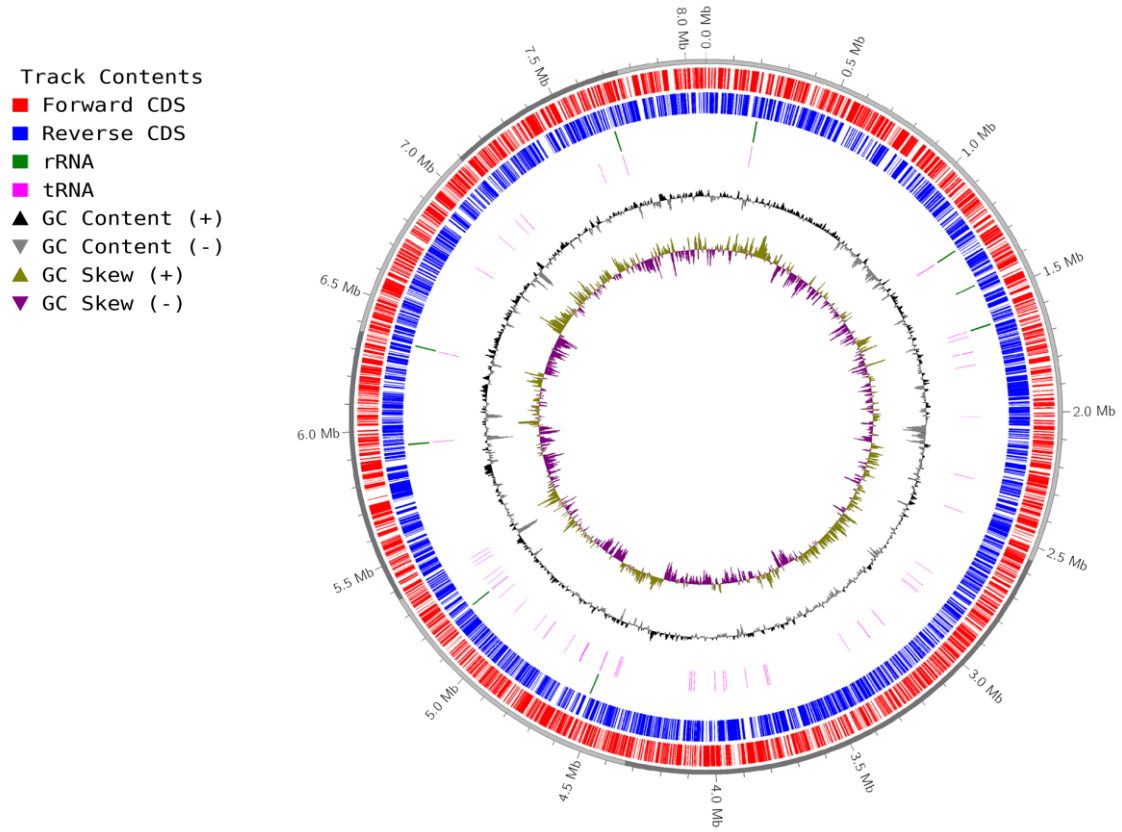

Fig 2. Genome map of the *NL1* strain.

According to Fig. 3, the current *Azospirillum* sp. NL1 strain was identified with the accession number of OR125575 and is close to the strain *Azospirillum lipoferum* NCIMB 11861^{T} (Xia et al., 1994) the most.

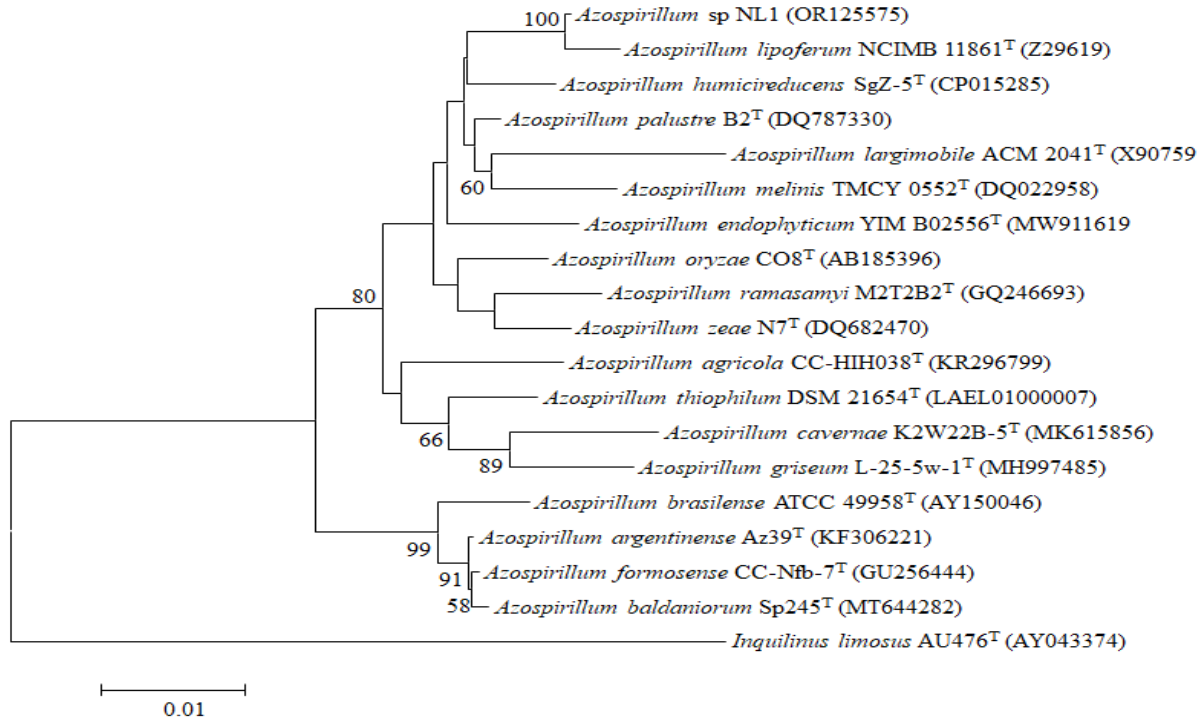

Fig 3. Evolutionary relationships of taxa.

Preliminary analysis of the functional genome structure capable of nitrogen fixation and IAA biosynthesis of Azospirillum sp. NL1

29 genes related to nitrogen fixation were identified based on the genome sequence of strain NL1. The results are presented in Table S6 and Fig. 9, with the genes nif and fix directly involved in the nitrogen fixation process. In addition, the genome analysis of strain NL1 also revealed 04 genes involved in the IAA metabolism pathway referenced on the MetaCyc database (Table S6 and Fig. 10).

Table S6 demonstrates that the *Azospirillum* sp. NL1 strain has 29 genes involving N_2 fixation, the pathway of which is illustrated in Fig. S6. The N_2 fixation of the *Azospirillum* sp. NL1 strain was performed by *nif* and *fix* genes. This agrees with the studies by Potrich et al. (2001) and Sperotto et al. (2004). The *Azospirillum* sp. NL1 strain also provides 4 genes involving IAA metabolism (Table S6 and Fig. S7). This is consistent with the study by Yusfi et al. (2004).

Materials and Methods

Bacterial isolation and selection

Because maize has been reported to contain NFB, soil was collected from maize fields in seven communes in Thai Nguyen province, Vietnam, including On Luong, Co Lung, Dong Dat, Hop Thanh, Phan Me, Yen Ninh, and Yen Trach. The soil was collected near maize plants at a depth of 0–20cm (Hou et al., 2023).

Ashby medium was used to determine NFB. The Ashby medium consisted of 20.0 g/L Mannitol, 0.2 g/L K_2HPO_4, 0.2 g/L $MgSO_4$, 0.2 g/L NaCl, 0.1g/L K_2SO_4, and 5.0 g/L $CaCO_3$ in distilled water (Wei et al., 2023). Bacterial strains that lived and grew in the Ashby medium were selected. The NH_4^+ production was measured in the Ashby broth at 30°C and shaken at 150 rpm for 7 days. The 7-day culture was then mixed with Nessler indicator and measured according to UV absorbance.

To determine IAA-producing NFB, the collected NFB were re-cultured in L-tryptophan (0.1%) – supplied Ashby medium. Since L-tryptophan is the precursor of IAA, IAA-producing bacteria can produce IAA in the medium containing L-tryptophan. Therefore, the method of Glickmann and Dessaux (1995) was used to determine IAA-producing NFB by changes in the color of the Salkowski indicator. The bacteria were cultured in Ashby medium (supplemented with Tryptophan 0.1%) at 30°C and shaken at 150 rpm for 7 days. The standard curve of standard IAA solutions reacting with the Salkowski indicator and being measured by a spectrophotometer served as the basis for the IAA production (Fig. S1).

Characterization of the selected IAA-producing NFB

Bacterial morphology

The morphology of the bacterial colonies was observed under a Scanning Electron Microscope (SEM) at a focal distance of 5 µm.

Response of the bacteria to temperature and pH

The bacterial culture had its temperature and pH adjusted and lasted for 5 days. The temperature ranged from 28°C to 42°C with 2°C intervals. The pH was from 4.0 to 9.0 with 0.5 intervals, which were obtained by the buffer solutions of Na_2HPO_4 and KH_2PO_4.

A spectrophotometer set to 610 nm wavelength was used to measure the bacterial density after five days of culture. From that, an optimal temperature and pH were calculated by the model of Box-Behnken with 17 combinations and 3 replications. The factors (-1, 0, and +1) included culture duration (A; 4, 5, and 6 days), the culture temperature (B; 30°C, 32°C, and 34°C), and culture pH (C; 6.0, 6.5, and 7.0). Thus, after the regression analysis, a multivariate model function of the cell density was obtained.

Enzyme production and biochemical functions by the bacteria

The enzyme production consisted of Phosphatase alkaline, Esterase (C4), Esterase Lipase (C8), Lipase (C14), Leucine arylamidase, Valine arylamidase, Cystine arylamidase, Trypsine, D-chymotrypsine, Acid phosphatase, Naphtol-AS-BI-phosphohydrolase, D-galactosidase, ß-galactosidase, ß-glucuronidase, D-glucosidase, ß-glucosidase, N-acetyl-ß-glucosaminidase, D-mannosidase, and D-fucosidase, and was tested by KIT API ZYM.

The biochemical functions consisted of Nitrate to nitrite conversion, Indole generation, D-glucose fermentation, L-arginine assimilation, Urea assimilation, Esculin ferric citrate assimilation, Gelatin assimilation, 4-nitrophenyl-β D-galactopyranoside assimilation, D-glucose assimilation, L-arabinose assimilation, D-mannnose assimilation, D-mannitol assimilation, N-acetyl-glucosamine assimilation, D-maltose assimilation, Potassium gluconate assimilation, Capric acid assimilation, Adipic acid assimilation, Malic acid assimilation, Trisodium citrate assimilation, Phenylacetic acid assimilation, L-rhamnose assimilation, D-ribose assimilation, Inositol assimilation, D-saccharose assimilation, Itaconic acid assimilation, Suberic acid assimilation, Sodium malonate assimilation, Sodium acetate assimilation, Lactic acid assimilation, L-alanine assimilation, Potassium 5-ketogluconate, Glycogen assimilation, 3-hydroxybenzoic acid assimilation, L-serine assimilation, Salicin assimilation, D-melibiose assimilation.

L-fucose assimilation, D-sorbitol assimilation, Propionic acid assimilation, Valeric acid assimilation, L-histidine assimilation, Potassium 2-ketogluconate assimilation, 3-hydroxybutyric acid assimilation, 4-hydroxybenzoic acid assimilation, L-proline assimilation, D-xylose assimilation, D-fructose assimilation, Lactose assimilation, Catalase activity, Oxidase activity, Methyl red reaction, Voges-Proskauer reaction, H_2S production. These functions were tested by the KIT API.

Genomic analysis of the bacteria

Crude sequences were purified to remove adapters, low-quality nucleotides, and repetitive sequences to gather the whole genome of the chosen bacteria. The selected bacterial culture was incubated for 2 days. Then, 2 mL of the culture was centrifuged for 5 min at 10,000 rpm and had its DNA extracted by a DNA extraction kit (Genomic DNA Prep Kit, BioFACT™). The extracted genomic DNA underwent a 1.0% w/v agarose gel electrophoresis and was checked under UV light for concentration and purity. The confirmed DNA was sequenced according to Whole Genome Sequencing via the next-generation sequencing by the Ktest sequencing service (Vietnam).

The raw sequencing data were quality-filtered using Fastp v0.23.1 (Chen et al., 2018). Nucleotides with poor sequencing quality, unreliable bases, or undetermined nucleotides (denoted as N) were removed based on the Phred quality scores (Illumina). The cleaned reads were subsequently assembled de novo using both Unicycler v0.4.8 (Wick et al., 2017) and the Flye–Medaka–Polca pipeline (Krasnov et al., 2020; Zimin and Salzberg, 2020). After comparing the outcomes of the two assembly techniques, the highest-quality assembly was chosen for taxonomic identification and annotation. The de novo assembly quality was assessed using QUAST v5.2.0 (Gurevich et al., 2013) and local read-to-contig alignments, which enabled the detection of regions with unusually low coverage depth compared to adjacent regions—an important indicator of potential assembly issues. CheckM v1.2.1 (Parks, et al., 2015) was employed to evaluate the quality and completeness of the assembly. The selected genome assembly was then annotated using the PATRIC annotation system v3.6.12 (Davis et al., 2020), which integrates a taxon-specific database for the target bacterial genus. PATRIC employs the RASTtk toolkit to annotate contig sequences. Taxonomic identification of the target bacterium was performed using GTDB-Tk v2.1.1 with the latest version of the Genome Taxonomy Database (GTDB), specifically curated for prokaryotes (Chaumeil et al., 2022; Jain et al., 2018).

Statistical analysis

The established model and the relationships between the variables influencing bacterial density were assessed using variance analysis (ANOVA). The expectation function method was applied to optimize the bacteria density obtained from the culture process by Design-Expert software (DX 7.1.5).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.22270683 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The Jukes-Cantor method was used to calculate the evolutionary distances (Jukes and Cantor, 1969), and they are in units of the number of base substitutions per site. 19 nucleotide sequences were analyzed. Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. The final dataset had 1183 positions in total. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

Conclusion

12 potent NFB candidates were isolated from 7 communes in Thai Nguyen, Vietnam. Among them, 8 isolates can synthesize IAA. The IAA and NH_4^+ productions were roughly 18.882–109.381 and 4.321–22.158 µg/ml, respectively. Therein, the NL1

strain showed the best performance. Thus, it was further characterized. The NL1 strain grew best under a duration of 5.22 days, temperature of 31.73°C, and pH of 6.61 to achieve a maximum cell density of 3.12159×10^8 CFU/ml as per the Box-Behnken-based growth function: $Y = +2.94 + 0.62*A + 0.023*B + 0.58*C + 0.12*A*B + 0.47*A*C - 0.15*B*C - 0.76*A^2 - 0.84*B^2 - 1.1*C^2$. Furthermore, the biochemical processes of the N1 strain were also detected. Moreover, the NL1 strain was identified as *Azospirillum* sp. with 12 N_2-fixing genes and 4 IAA-producing genes. Therefore, the *Azospirillum* sp. NL1 strain is promising to be a good N_2 fixer and IAA producer for ameliorations in soil fertility and crop productivity, which can ultimately replace a portion of chemical fertilizer for sustainable agriculture.

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Statement of contributions
TVC and NXB were responsible for the conceptualization and writing of the first draft. NTD, NMT, and NTH conducted biochemical experiments. PTTM, NTG, NTHA, and HTLA isolated the bacterial strains and conducted genomic analysis. NQK involved methodology, reviewing, and editing the manuscript. All authors commented and approved the final version of the manuscript.

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