JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2026, 27(6), 97–113
https://doi.org/10.12911/22998993/216323
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.12.24
Accepted: 2026.04.16
Published: 2026.05.01

Isolation and selection of rhizosphere bacteria associated with *Limnocharis flava* grown in a hydroponic system for biofilm biofertilizer development

Rizka Novi Sesanti[1,2], Edi Purwanto[3*], Samanhudi[3], Sudadi[4]

[1] Doctoral Program of Agricultural Science, Sebelas Maret University, Jl. Ir Sutami 36 A Kentingan, Surakarta, Indonesia
[2] Study Program of Horticulture Production Technology, Department of Food Crop Cultivation, Politeknik Negeri Lampung, Jl. Soekarno Hatta, No. 10, Rajabasa, Bandar Lampung, Lampung, Indonesia
[3] Department of Agricultural Science, Faculty of Agriculture, Sebelas Maret University, Jl. Ir Sutami 36 A, Kentingan, Surakarta, Indonesia
[4] Department of Soil Science, Faculty of Agriculture, Sebelas Maret University, Jl. Ir Sutami 36 A, Kentingan, Surakarta, Indonesia
* Corresponding author's e-mail: edipurwanto@staff.uns.ac.id

ABSTRACT

The development of sustainable hydroponic systems requires utilization of plant microbe, particularly those involving rhizosphere bacteria with plant growth promoting traits. This study aim to the isolation and selection of indigenous rhizosphere bacteria with plant growth-promoting properties for the development of biofilm-based biofertilizers in hydroponic systems. Bacteria were isolated from the rhizosphere of Limnocharis flava grown under different substrate combinations and nutrient concentrations using serial dilutions on Nutrient Agar. A total of 23 isolates were characterized through morphological, physiological, biochemical, and functional tests, including nitrogen fixation, phosphate and potassium solubilization, indole-3-acetic acid (IAA) production, and biofilm formation. Quantitative evaluation of N fixation, P and K solubilization, and IAA production was performed using UV-Vis spectrophotometer and AAS. The isolates showed considerable diversity, and three superior strains were identified: A34 (high potassium solubilization and IAA production), J31 (stable biofilm formation in both single and consortium cultures), and M13 (highest nitrogen fixation and phosphate solubilization). These results indicate that the rhizosphere of L. flava is a promising source of native bacteria for the development of biofilm-based biofertilizers in sustainable hydroponic cultivation.

Keywords: sustainable, hydroponic, substrate, nutrient, bacterial, plant growth promoting bacteria.

INTRODUCTION

Biofilm biofertilizer is a biofertilizer that contains a consortium of functional microbes capable of forming a biofilm layer. This layer consists of extracellular polymers that can break microbial dormancy to function optimally through biochemical signals between plants, microbes, and soil fauna (Premarathna et al., 2021). Brokate et al. (2024) reported that biofilm-forming microorganisms create a protective microenvironment in the rhizosphere, thereby supporting plant growth even under abiotic stress conditions. Similarly, Rafique et al. (2024) showed that biofilm forming PGPRs are capable of promoting more efficient root colonization and cooperative interactions with plants, thereby increasing nutrient uptake and yield stability. In leafy vegetables, a combination of 48% chemical fertilizer and biofilm biofertilizer produced yields equivalent to those obtained with 100% chemical fertilizer, enabling a 52% reduction in chemical fertilizer use without compromising productivity (Gangthilaka et al., 2022). In tomato plants, inoculation with biofilm biofertilizer increased plant height by 49.14%, fresh shoot weight by 32.47%, and root length by 45.00% compared to the control without inoculation (Kaundal et al., 2025). These findings are consistent with Sarti et al. (2024), who reported that biofilm biofertilizer inoculation significantly increased tomato yield, as reflected in the increase in fruit number and weight, compared to no inoculation.

Conventionally, bacterial isolates used for biofilm biofertilizer development are predominantly sourced from agricultural soils or the rhizosphere of soil-grown crops (Amalina et al., 2023). In contrast, microbial resources associated with hydroponic cultivation have received comparatively little attention, largely due to the longstanding perception of hydroponic systems as microbially simplified or quasi-sterile environments. However, accumulating evidence indicates that hydroponic substrates can support diverse and metabolically active rhizosphere bacterial assemblages that establish intimate interactions with plant roots (Stegelmeier et al., 2022). While the functional importance of rhizosphere bacteria in soil-based agriculture is well documented, their ecological roles and applied potential within hydroponic systems remain insufficiently characterized. As a result, current hydroponic research has been predominantly plant-centric, with microbial dimensions often treated as secondary considerations (Banboukian et al., 2025).

Limnocharis flava is an aquatic leafy vegetable that is widely consumed in Southeast Asia. *L. flava* usually grows in wetlands or waterlogged environments (Putra et al., 2023). *L. flava* has a habitat similar to that of a substrate hydroponic system. Substrate hydroponics is a hydroponic system that uses growing media other than soil, more commonly known as substrates. Aquatic plants such as *L. flava* are often associated with unique rhizosphere microbial communities that are adapted to submerged or semi submerged conditions. Supporting this assumption, microbial inoculation studies have demonstrated that *L. flava* associated bacteria can enhance plant growth and improve tolerance to heavy metal stress, particularly cadmium contamination (Lam et al., 2022). Comparable observations in other aquatic macrophytes, including Vallisneria natans and Lemna minor, further suggest that aquatic plant rhizospheres represent promising reservoirs of plant growth-promoting bacteria with functional traits that are less frequently reported in terrestrial plant systems (Makino et al., 2022; Schmautz et al., 2021; Wang et al., 2021). In parallel, recent hydroponic studies have revealed that controlled soilless systems may selectively enrich beneficial bacterial groups, such as Rhodanobacter, Chujalbacter, and Thermomonas, which are associated with improved nutrient use efficiency and plant performance (Alkaabi et al., 2025; Chowdhury and Samarakoon, 2024; Spencer et al., 2024).

Although research on rhizosphere bacteria in aquatic plants has been conducted, most studies are still limited to phytoremediation functions or interactions in natural ecosystems (Goulart et al., 2024; Sun et al., 2025). To date, research discussing the isolation and selection of *L. flava* rhizosphere bacteria with potential as biofilm biofertilizers in hydroponic conditions is still limited. Therefore, this study aims to isolate, characterize, and select rhizosphere bacteria associated with *L. flava* in hydroponic systems for the development of biofilm biofertilizers. These findings are expected to contribute not only to knowledge of microbial biodiversity but also to the development of biofilm biofertilizers in hydroponic systems, thereby supporting sustainable hydroponic cultivation systems.

MATERIALS AND METHODS

Study area

This research was conducted at the Soil Biology and Biotechnology Laboratory, Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java, Indonesia, from October 2024 to July 2025. Rhizosphere bacteria were isolated from substrates around the roots of *L. flava* plants cultivated using a hydroponic system. Substrate samples were collected in the hydroponic greenhouse of the Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia, in October 2024 (altitude 131 m above sea level; coordinates 7°33′41.8″ S and 110°51′32.36″ E)

Sample preparation

Isolate samples were taken from the rhizosphere of *L. flava* grown in a hydroponic system on various types of substrates and nutrient concentrations. The substrates used were volcanic sand, rice husk charcoal, and a mixture of volcanic sand + rice husk charcoal (1:1) combined with nutrient solution concentrations of 0, 0.8, 1.6, 2.4, and 3.2 dS m^{-1}. The variations in substrate type and nutrient concentration were not treated as experimental treatments, but were only used as sources of variation in rhizosphere conditions. This approach aimed to obtain bacterial isolates that reflected the diversity of the *L. flava* rhizosphere bacterial community in substrate hydroponic systems. The nutrient formula was designed to provide 180 ppm NO_3^-, 37 ppm NH_4^+, 66 ppm P, 286 ppm K, 154 ppm Ca, 66 ppm Mg, and 122 ppm S, supplemented with 40 g of Vitaflex™ micronutrient mix in 5 L of concentrated stock solution. The samples were placed in labeled plastic bags, then put in a coolbox and transported to the laboratory.

Isolation of rizosfer bacteria from *L. flava*

Bacterial isolation was performed by diluting 10 g of substrate sample in 90 mL of physiological saline, followed by thorough mixing until homogeneous. The suspension was subsequently subjected to serial dilutions up to 10^{-9} and homogenized using a vortex mixer. The 10^{-5} dilution was selected for bacterial isolation. A 0.1 ml of the diluted suspension was transferred using a micropipette and spread onto nutrient agar (NA) plates using a Drigalski spatula following the spread-plate method (Pujiati et al., 2025). Each treatment was conducted in duplicate. The plates were then incubated for five days.

Characterization of rizosfer bacteria from *L. flava*

Colony morphology (color, diameter, shape, margin, elevation, and internal structure) was assessed macroscopically (Linda et al., 2023; Masi et al., 2021). Microscopic characteristics and Gram reaction were determined using Gram staining (Oktari et al., 2021). Aerobicity was evaluated in Nutrient Broth based on growth patterns under oxygen availability. Catalase activity was assessed using H_2O_2, indicated by bubble formation (Sudewi et al., 2020). Biochemical tests included Simmons Citrate Agar (SCA) and Triple Sugar Iron Agar (TSIA) assays, with color changes interpreted as described by (Goa et al., 2022). Bacterial motility was determined on NA medium following vertical inoculation.(Martínez-Cámara et al., 2020).

Screening for nitrogen fixation

Nitrogen fixation was evaluated using qualitative and quantitative approaches. Qualitative screening was conducted by culturing bacterial isolates on nitrogen-free Jensen medium and incubating them at room temperature for 7 days; colony growth indicated potential nitrogen fixation. Quantitative analysis was performed by incubating isolates in liquid peptone medium under shaking conditions (70 rpm) for 5 days. Cultures were centrifuged (4000 rpm, 10 min), and ammonium production in the supernatant was determined using the Nessler method, with absorbance measured at 420 nm using a UV–Vis spectrophotometer. NH_4^+ concentrations were calculated based on an NH_4Cl standard curve (Li et al., 2024; Mondal et al., 2024; Shi et al., 2023).

Screening for phosphate solubilization

Phosphate solubilization was first assessed qualitatively by inoculating isolates onto Pikovskaya agar and incubating them at room temperature for 7 days. The formation of clear halos around colonies indicated phosphate solubilization. Quantitative determination was conducted in Pikovskaya liquid medium incubated under shaking (70 rpm) for 5 days. After centrifugation (1.000 rpm, 15 min), soluble phosphate in the supernatant was measured colorimetrically using the molybdenum blue method, with absorbance recorded at 882 nm. Phosphate concentrations were calculated using a KH_2PO_4 calibration curve (Aliyat et al., 2022).

Screening for potasium solubilization

Potassium solubilization was evaluated qualitatively on Aleksandrov agar supplemented with feldspar as the sole potassium source, following incubation at room temperature for 7 days. Halo formation around colonies indicated solubilization activity. Quantitative analysis was performed by culturing isolates in Aleksandrov liquid medium containing feldspar for 7 days under shaking conditions (70 rpm). After centrifugation (2.500 rpm, 25 min) and filtration, dissolved potassium in the supernatant was quantified using atomic absorption spectroscopy (AAS) at 766.5 nm, with KCl used as the standard (Nguyen et al., 2024)

Screening for IAA production

IAA production was assessed by growing isolates in nutrient broth supplemented with L-tryptophan (0.1 g L^{-1}) for 48 h under shaking conditions (70–80 rpm). Following centrifugation (11,000 rpm, 15 min), the supernatant was reacted with Salkowski reagent (1:1, v/v) and incubated in the dark for 30 min. Development of a pink to purplish-red color indicated IAA production. Quantitative IAA levels were determined spectrophotometrically at 530 nm using an IAA standard curve (Gen-jiménez et al., 2023; Lebrazi et al., 2020).

Screening for the biofilm forming ability

Biofilm formation was qualitatively evaluated using the tube assay. Isolates were incubated in nutrient broth for 4 days at room temperature with shaking (70 rpm). Tubes were then washed, stained with 0.1% crystal violet, and rinsed with phosphate-buffered saline. The presence of a violet film adhering to the tube wall was interpreted as positive biofilm formation (Basnet et al., 2023).

Data analysis

Data analysis was performed on the quantitative potential to bind N, solubilize P and K, and produce IAA. All treatments were replicated three times. Data were analyzed using one-way ANOVA. Differences between treatments were considered significant at $p < 0.05$. SPSS 26 and Excel.

RESULTS AND DISCUSSION

Result

Isolation and characteristics of L. flava rhizosphere bacteria cultivated using a hydroponic system

Isolation of *L. flava* rhizosphere bacteria under various microhabitat conditions formed from a combination of substrate types (volcanic sand, charcoal husks, and a mixture of both) and varying concentrations of nutrient solutions (0, 0.8, 1.6, 2.4, and 3.2 dS m^{-1}) resulted in 23 bacterial colonies. The selected isolates were coded A34, B22, B31, B34, C13, C24, D12, E12, F21, G14, G22, G31, H11, I31, J12, J31, K31, L31, M13, M15, N11, N21, and O21.

The morphological characteristics of these rhizosphere bacteria were examined through macroscopic and microscopic observations (Table 1). The results of the observation showed that there were various colors of colonies, such as milky white, cream, yellow, orange, pink, brick red, and red. Some isolated colonies, particularly B22, G22, G31, H11, K31, and M15, had color pigments. Colony diameters varied between 0.55 and 1.70 mm. The identified colony shapes were predominantly circular, while there were also irregular (C13, D12, F21, and L13), myceloid (J31), and spindle (K31) shapes. Five different colony edge shapes were identified: undulate (A34, B22, B34, C24, G22, G31, I31, and K31), fimbriate (B31, C13, and J31), entire (D12, J12, L13, and M15), erose (E12, F21, H11, M13, and N11), and lobate (G14, N21, and O21). Meanwhile, the elevation profile showed high diversity, including umbonate, low convex, convex rugose, convex papillate, raised, and effuse forms. Similarly, the internal colony structure was highly variable, including finely granular, opaque, filamentous, wavy entrapped, translucent, coarsely granular, arborescent, and smooth textures. No isolates showed identical combinations of margin, height, and internal structure, reflecting substantial morphological diversity among rhizosphere bacterial communities associated with *L. flava* in hydroponic culture. The cell shape was mostly basil, and only isolates I31 and J12 showed coccus and coccobacillus cell shapes. Gram staining results mostly showed that the isolates observed were gram negative, and only 5 isolates were gram positive, namely isolates K31, L13, M13, N11, and N21.

The results of physiological and biochemical testing of 23 rhizosphere bacterial isolates of *L. flava* showed variations in characteristics that reflect the metabolic diversity of bacteria in substrate based hydroponic systems (Table 2).

Most isolates were aerobic, indicating that the root zone of *L. flava* in this system provides adequate oxygen conditions for aerobic respiration. Several isolates, such as A34, C24, J12, and N11, were facultative aerobic, indicating flexibility in utilizing conditions with or without oxygen, while only a few were anaerobic. The catalase test showed positive results in all isolates, indicating the universal capacity of the rhizosphere bacterial community to detoxify hydrogen peroxide accumulation, which is commonly found in active root environments. Variations began to appear in the citrate utilization test, with several isolates able to use citrate as a carbon source,

Table 1. Macroscopic and microscopic characteristics of 23 rhizosphere bacterial colonies of *L. flava* cultivated in a hydroponic system

Isolat code	Macroscopic characteristics						Microscopic characteristics	
	Color	Diameter (mm)	Colony shape	Colony margin	Elevation	Opacity	Cell	Gram staining
A34	Cream	1.10	Circular	Undulate	Umbonate	Finely granular	Bacil	-
B22	Yellow	1.00	Circular	Undulate	Law conves	Opaque	Bacil	-
B31	Milky white	1.15	Circular	Fimbriate	Convex rugose	Filamentous	Bacil	-
B34	Cream	0.90	Circular	Undulate	Umbonate	Finely granular	Bacil	-
C13	Cream	1.70	Irregular	Fimbriate	Umbonatelaw convex	Wavy enteriaced	Bacil	-
C24	Cream	1.00	Circular	Undulate	Law convex	Opaque	Bacil	-
D12	Cream	1.00	Irregular	Entire	Law convex	Coarsely granular	Bacil	-
E12	Milky white	1.10	Circular	Erose	Convex papilate	Transculent	Bacil	-
F21	Milky white	1.10	Irregular	Erose	Convex rugose	Coarsely granular	Bacil	-
G14	Cream	0.95	Circular	Lobate	Convex papilate	Finely granular	Bacil	-
G22	Pink	1.10	Circular	Undulate	Convex papilate	Arborescent	Bacil	-
G31	Orange	0.55	Circular	Undulate	Umbonate	Finely granular	Bacil	-
H11	Red	0.55	Circular	Erose	Convex papilate	Transculent	Bacil	-
I31	Milky white	1.00	Circular	Undulate	Law convex	Opaque	Coccus	-
J12	Cream	1.10	Circular	Entire	Law convex	Coarsely granular	Coccobacillus	-
J31	Cream	1.10	Myceloid	Fimbriate	Raised	Wavy enteriaced	Bacil	-
K31	Brick red	1.20	Spindle	Undulate	Law convex	Smooth	Bacil	+
L13	Milky white	1.00	Irregular	Entire	Law convex	Coarsely granular	Bacil	+
M13	Cream	1.00	Circular	Erose	Convex papilate	Tranculent	Bacil	+
M15	Yellow	1.10	Circular	Entire	Effuse	Opaque	Bacil	-
N11	Milky white	1.00	Circular	Erose	Law convex	Wavy enteriaced	Bacil	+
N21	Cream	1.10	Circular	Lobate	Convex papilate	Coarsely granular	Bacil	+
O21	Milky white	1.10	Circular	Lobate	Convex papilate	Coarsely granular	Bacil	-

namely isolates with codes A34, B31, B34, E12, F21, G14, G22, G31, L13, and M15, while other isolates showed a negative response. The same differences were also seen in the TSIA test, with some isolates showing the ability to ferment sugar or produce H2S, while others tended to be non-fermentative. This diversity shows metabolic function differentiation that is relevant to the nutritional dynamics in the hydroponic root zone. The motility characteristics of the isolates also varied, with a number of isolates such as B22, C13, E12, G22, G31, I31, J12, K31, L13, M15, N11, and N21 exhibiting motile characteristics, while other isolates were nonmotile.

Qualitative and quantitative analysis in fixing N

A total of seven bacterial isolates, namely I31, B22, O21, C24, K31, M13, and G31 (Figure 1), showed the ability to grow on Jensen medium (N free medium), a condition that generally limits the growth of bacteria that cannot fix N.

The ability to survive on this nitrogen deficient medium reflects the presence of physiological mechanisms that enable the isolates to fix nitrogen directly. Successful growth under selective conditions shows that this group of isolates is not only adaptive, but also has the

Table 2. Physiological and biochemical characteristics of 23 rhizosphere bacterial isolates of *L. flava* cultivated in a hydroponic system

Isolat Code	Aerobicity	Catalase	SCA test (*cimmons citrate Agar*)	TSIA test (*triple sugar Iron Agar*)	Motility test
A3 4	Aerob fakultatif	+	+	-	-
B2 2	Aerob	+	-	+	+
B3 1	Aerob	+	+	+	-
B3 4	Aerob	+	+	+	-
C1 3	Aerob	+	-	-	+
C2 4	Aerob fakultatif	+	-	+	-
D1 2	Aerob	+	-	+	-
E1 2	Aerob	+	+	-	+
F2 1	Aerob	+	+	+	-
G1 4	Aerob	+	+	+	-
G2 2	Anaerob	+	+	+	+
G3 1	Anaerob	+	+	-	+
H1 1	Aerob	+	-	+	-
I3 1	Aerob	+	-	+	+
J1 2	Aerob fakultatif	+	-	+	+
J3 1	Aerob	+	-	-	-
K3 1	Aerob	+	-	+	+
L1 3	Aerob	+	+	+	+
M1 3	Aerob	+	-	+	-
M1 5	Aerob	+	+	+	+
N1 1	Aerob fakultatif	+	-	+	+
N2 1	Aerob	+	-	+	+
O2 1	Aerob	+	-	+	-

potential to play an important ecological role in nitrogen supply in the root zone of hydroponically cultivated *L. flava*.

Quantitative analysis of nitrogen fixation ability using a UV Vis spectrophotometer (Table 3) showed that all seven bacterial isolates produced nitrogen accumulation in the form of ammonium (NH_4^+) with fairly clear variations in response between isolates. Isolate M13 showed the highest value, namely 26.72 ± 0.04 mg l^{-1}, indicating the most intensive nitrogen fixation activity compared to other isolates. Isolates C24, G31, I31, K31, and O21 showed similar values, while isolate B22 gave the lowest value of 23.58 ± 0.65 mg l^{-1}. However, these isolates still possessed nitrogen fixation capabilities. Overall, this quantitative pattern reinforces the evidence that the seven isolates have physiological potential in producing fixed nitrogen and are worthy of consideration as nitrogen-fixing bacterial candidates for biofertilizer applications in substrate based hydroponic systems.

Qualitative and quantitative analysis in dissolving phosphate

Five bacterial isolates (M15, M13, J12, N21, and L13) showed the ability to dissolve phosphate, as detected by the appearance of clear zones on Pikovskaya medium (Figure 2). This may indicate that the five isolates are capable of breaking down bound inorganic phosphate into a more available form. The difference in the width of the dissolution zone suggests that the metabolic response between isolates is not homogeneous, and some of them show higher effectiveness in mobilizing phosphate. These results indicate that only a small fraction of the selected bacterial community has a strong functional capacity to increase phosphorus availability in the root environment. These findings position these five isolates as potential candidates for further development in P-solubilizing biofertilizer formulations for *L. flava*.

Measurement of dissolved phosphate levels using a UV Vis spectrophotometer showed that

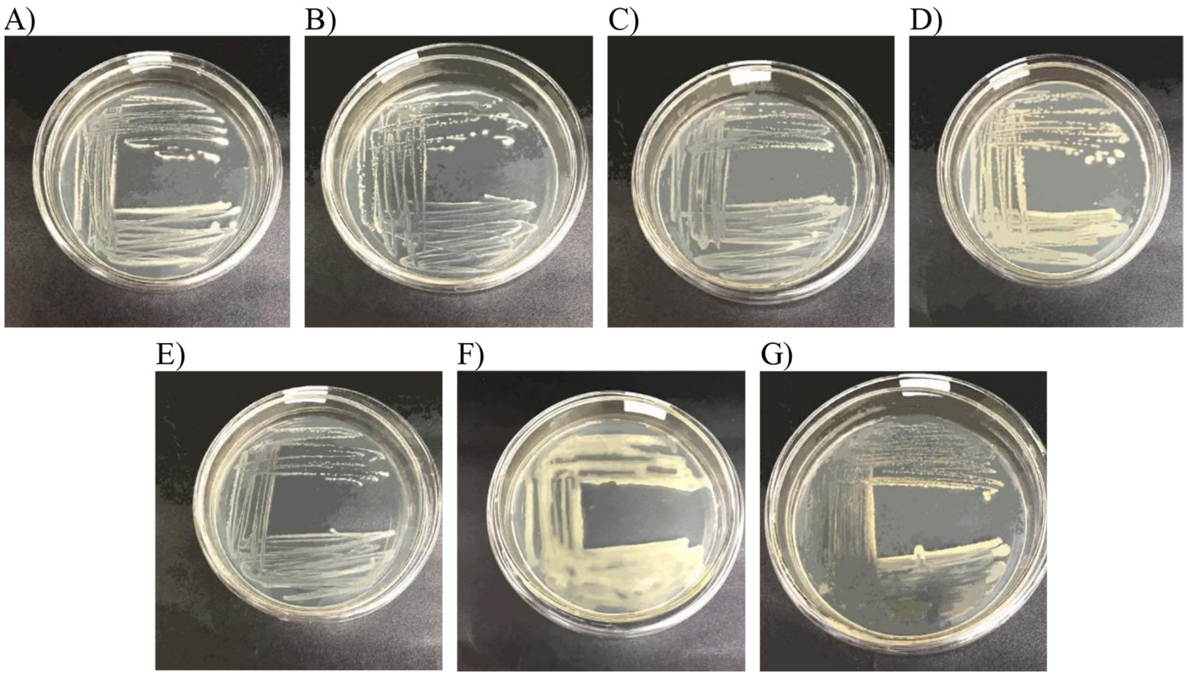

Figure 1. Identified bacterial isolates have nitrogen-fixing capabilities. (A) I31, (B) B22, (C) O21, (D) C24, (E) K31, (F) M13, (G) G31

Table 3. Results of quantitative analysis of nitrogen fixation ability using a UV Vis spectrophotometer

Isolat code	Concentration NH_4(mg l^{-1})
B22	23.58 ± 0.65 b
C24	24.98 ± 1.85 ab
G31	24.55 ± 1.47 ab
I31	24.99 ± 1.85 ab
K31	25.22 ± 0.16 ab
M13	26.72 ± 0.04 a
O21	25.07 ± 1.92 ab
CV%	5.49

Note: The numbers followed by the same letters and columns showed no significant difference based on the one way Anova at a level of 5%.

the five isolates had different abilities to dissolve P (Table 4). Isolate M13 had the highest ability, namely 6.17 ± 0.42 mg l^{-1}, reflecting a stronger dissolution efficiency compared to other isolates, but statistically not different from isolates N21 and M15 (5.78 ± 0.29 mg l^{-1} and 5.69 ± 0.16 mg l^{-1}). Meanwhile, isolates L13 (5.47 ± 0.01 mg l^{-1}) and J13 (5.09 ± 0.34 mg l^{-1}) showed the lowest P solubilization capacity compared to other isolates. These results indicate a gradient in phosphate solubilization ability among the isolates tested, but remain relevant in the context of P-solubilizing microbial function.

Qualitative and quantitative analysis in dissolving K

Of the 23 isolates tested for their ability to dissolve K using Alexandrov's specific medium, six isolates (M13, L13, B22, A34, E12, and D12) showed the ability to dissolve potassium. This was indicated by the formation of a clear zone around the colonies on Alexandrov's medium (Figure 3). The clear zones in the six isolates confirmed that all of them were capable of mobilizing potassium from insoluble mineral sources. However, the clear zones produced varied in size, ranging from thin circles to wider zones.

Quantitative testing of the ability to dissolve K in six bacterial isolates showed quite clear variations in response between isolates. Isolate A34 displayed the highest dissolution capacity with a value of 1.86 ± 0.15 mg l^{-1}, marking the strongest performance among all isolates tested. Isolate M13 was next with a value of 1.58 ± 0.07 mg l^{-1}, followed by three other isolates, namely D12, B22, and L13, which showed relatively similar dissolution values, namely 1.41 ± 0.05 mg l^{-1}, 1.40 ± 0.04 mg l^{-1}, and 1.42 ± 0.11 mg l^{-1}, respectively. Meanwhile, isolate E12 showed the lowest solubility value, namely 1.31 ± 0.06 (Table 5).

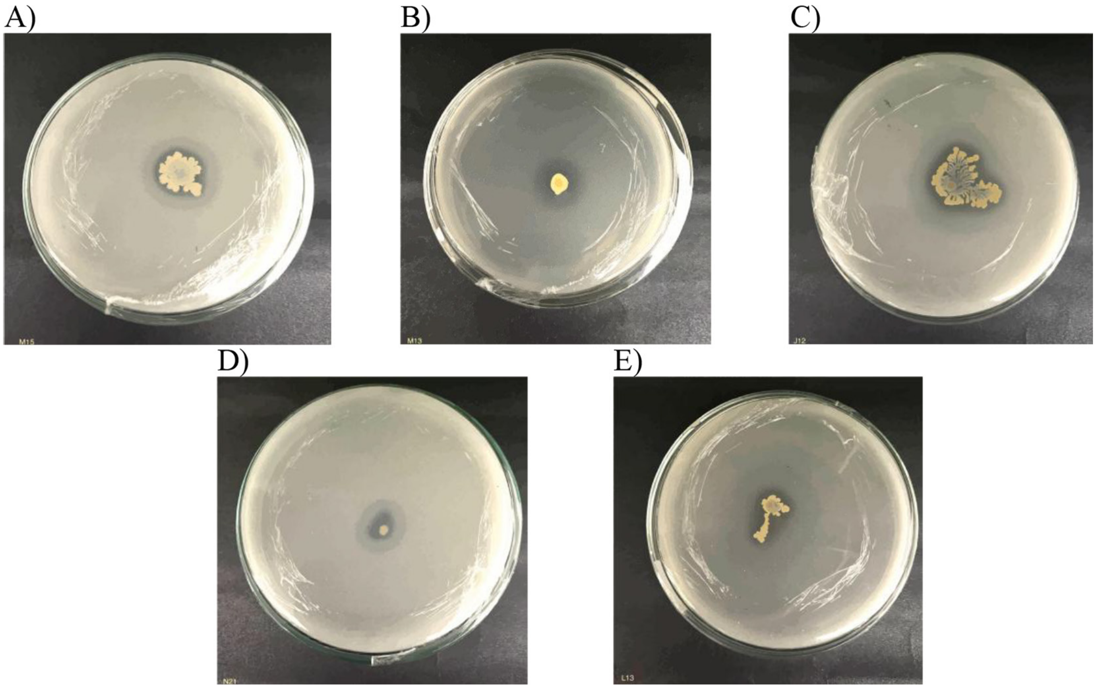

Figure 2. Identified bacterial isolates have the ability to dissolve phosphate. (A) M15, (B) M13, (C) J12, (D) N21, (E) L13

Table 4. Results of quantitative analysis of phosphate solubility using a UV Vis spectrophotometer

Isolat code	Phosphate solubilization value (mg l^{-1})
J13	5.09 ± 0.34 c
L13	5.47 ± 0.01 bc
M13	6.17 ± 0.42 a
M15	5.69 ± 0.16 ab
N21	5.78 ± 0.29 ab
CV%	5.07%

Note: The numbers followed by the same letters and columns showed no significant difference based on the one way anova at a level of 5%.

Table 5. Results of quantitative analysis of potassium solubility using AAS

Isolat code	Potasium solubilization value (mg l^{-1})
A34	1.86 ± 0.15 a
B22	1.40 ± 0.04 bc
D12	1.41 ± 0.05 bc
E12	1.31 ± 0.06 c
L13	1.42 ± 0.11 bc
M13	1.58 ± 0.07 b
CV%	6.17%

Note: The numbers followed by the same letters and columns showed no significant difference based on the one way anova at a level of 5%.

Qualitative and quantitative analysis in the production of IAA

Qualitative test results for indole-3 acetic acid (IAA) production show that several bacterial isolates (A34, B31, D12, G31, K31, and M13) from the rhizosphere of *L. flava* are capable of producing IAA. This was indicated by a change in the color of the culture suspension to pink after the addition of Salkowski reagent (Figure 4). Variations in color intensity between isolates showed differences in IAA production capacity, with some isolates displaying a stronger pink hue than others. This phenomenon indicates that the potential for IAA synthesis is not uniform among the isolates tested (Table 6).

Quantitative measurements were performed on six isolates suspected of producing IAA, namely isolates A34, B31, D12, G31, K31, and M13. Among all isolates tested, isolate A34 showed the highest IAA production, reaching 26.90 ± 0.32 mg l^{-1}. Meanwhile, the other five isolates, B31, D12, G31, K31, and M13, showed IAA concentrations in the same range (22.90–23.75 mg l^{-1}). Overall, this quantitative pattern confirms the existence of metabolic capacity differences between isolates, with one superior isolate surpassing the others, making it a candidate isolate for producing biofilm biofertilizer.

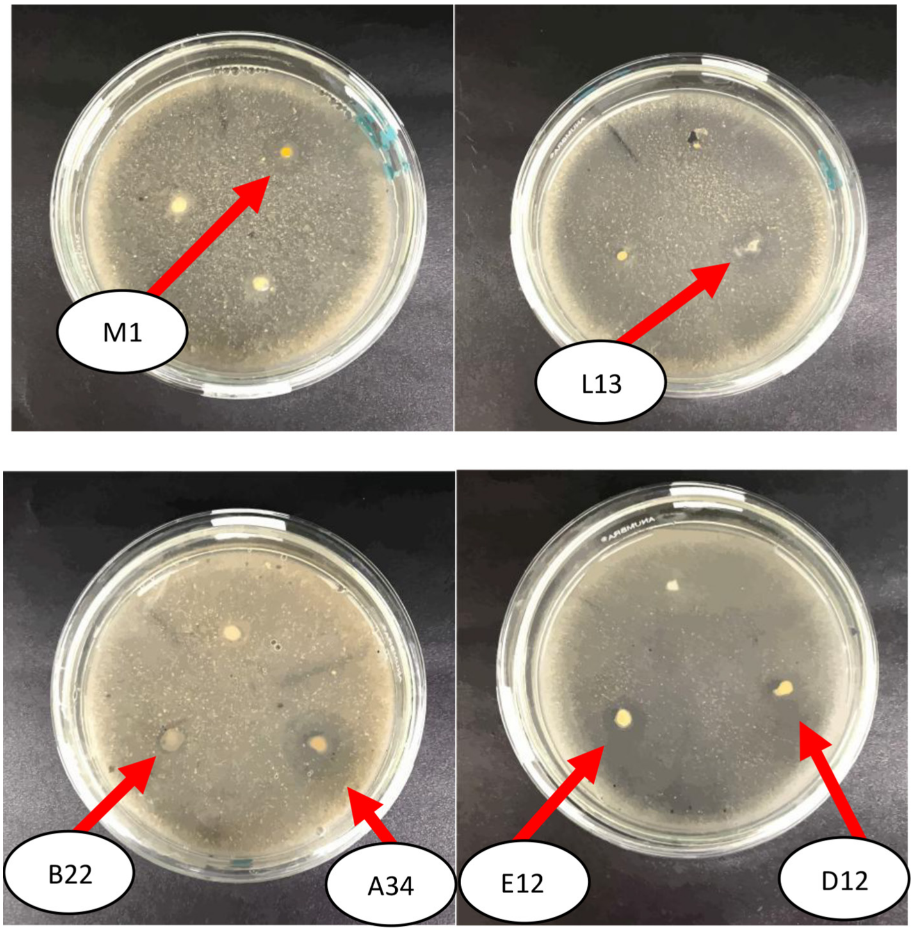

Figure 3. Identified bacterial isolates have the ability to dissolve potassium

Biofilm

The results of biofilm formation analysis showed that only a small portion of isolates were capable of producing biofilm structures under the test conditions (Figure 5).

Of the total 23 isolates tested, only two isolates showed biofilm formation, namely isolates J31 and J12. A positive biofilm test was indicated by the appearance of a purple film layer adhering to the tube wall on the surface of the culture after the staining process. These two isolates showed stronger adhesion and matrix accumulation abilities compared to the other isolates, which did not show any film layer at all.

Discussion

Isolation and characterization of rhizosphere bacteria L. flava

Isolation of *L. flava* rhizosphere bacteria under different microhabitats formed by combinations of substrate types and nutrient concentrations resulted in substantial morphological colony diversity, consistent with reports from other hydroponic systems showing that substrate and nutrient variation shapes distinct bacterial communities (Gerrewey et al., 2021; Guevara et al., 2025). A total of 23 colonies were obtained, characterized morphologically (macroscopically and microscopically). Macroscopic and microscopic characteristics including color, diameter, shape, margin, elevation, internal structure, cell shape, and gram reaction were then examined (Sheikh et al., 2024). Colony colors ranged from milky white to red, with several isolates likely producing pigments. Pigmented bacteria such as Chryseobacterium and Bacillus subtilis are known for functional metabolites including carotenoids and pulcherrimin, which enhance stress tolerance and provide antimicrobial activity (Orlandi et al., 2022; Salo and Novero, 2020). *Serratia* spp. are likewise known for producing prodigiosin with biocontrol potential (Soenens and Imperial 2020). Macroscopic traits varied extensively among isolates, consistent with environmental

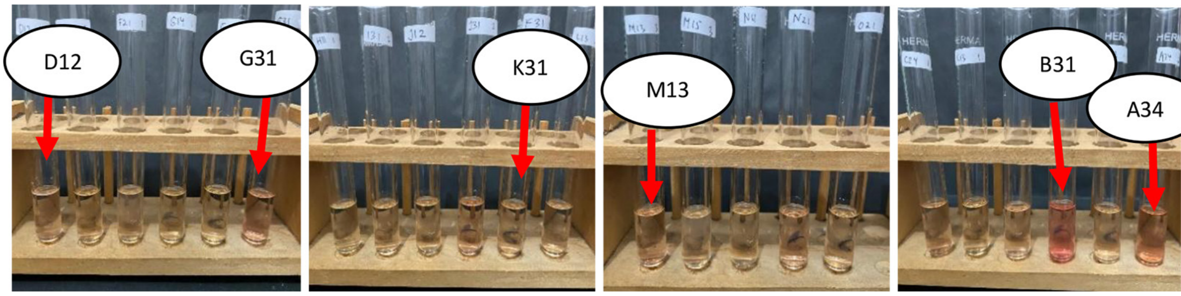

Figure 4. Qualitative test results for indole-3 acetic acid (IAA) production by bacterial isolates due to the addition of Salkowski reagent

Table 6. Results of quantitative analysis of IAA production capacity

Kode isolate	IAA (mg l^{-1})
A34	26.90 ± 0.32 a
B31	22.90 ± 2.40 b
D12	23.12 ± 1.40 b
G31	23.34 ± 1.89 b
K31	23.17 ± 1.06 b
M13	23.75 ± 0.67 b
CV%	6.17%

Note: The numbers followed by the same letters and columns showed no significant difference based on the one way anova at a level of 5%.

Figure 5. Identified bacterial isolates have the ability to form biofilms under single conditions with crystal violet staining

and strain-dependent influences on bacterial morphology (Sousa et al., 2013).

Microscopic examination showed predominantly bacillary forms, with few cocci and coccobacilli. Gram staining revealed that most isolates were Gram-negative, with only five Gram-positive. These characteristics reflect differences in cell wall structure and ecological strategies; both Gram-positive and Gram-negative groups may exhibit PGPR traits (Hardiansyah et al., 2020; Samet et al., 2022). Such morphological variation suggests potential functional diversity within the community (Chhetri et al., 2025). Most isolates were aerobic, while some were facultatively aerobic, aligning with previous findings that genera such as Bacillus can tolerate variable oxygen conditions (Crivelli et al., 2024). All isolates displayed positive catalase activity, indicating the ability to degrade H_2O_2 and tolerate oxidative stress an important trait for biofertilizer application (Khatoon et al., 2022). The SCA test confirmed the ability of several isolates to utilize citrate, a trait linked to enhanced phosphate solubilization and improved nutrient uptake in plants (Goa et al., 2022; Wagh et al., 2014). TSIA results demonstrated the capacity to ferment different sugars, reflecting metabolic versatility relevant to root exudate utilization and PGPR performance (P. Shi et al., 2022). Motility tests showed variable mobility, a trait associated with better colonization and biofilm establishment in root environments, as exemplified by *Bacillus ginsengihumi* M2.11 (Itkina et al., 2025; Martínez-Cámara et al., 2020).

Overall, the physiological and biochemical profiles demonstrate not only structural but also functional diversity among isolates, driven by hydroponic microhabitat variation. These findings highlight the adaptive potential of the rhizosphere microbiome and provide a strong basis for selecting promising isolates for the development of biofilm-based biofertilizers in hydroponic systems.

The potential of L. flava rhizosphere bacterial isolates in fixing N, solubilizing P and K, producing IAA, and forming biofilms

Seven isolates (I31, B22, O21, C24, K31, M13, and G31) were able to grow on Jensen medium, indicating their ability to maintain metabolism under minimal nitrogen conditions and suggesting potential nitrogenase activity, consistent with reports that survival on N-free medium reflects active nif gene expression (Bennett et al., 2023). This ability is ecologically valuable in substrate based hydroponics, where organic nitrogen reserves are limited. Quantitative measurements using UV–Vis spectrophotometry showed that M13 had the highest nitrogen-fixing capacity, supporting findings that fixation efficiency varies among strains due to nitrogenase regulation and adaptation to root-zone oxygen conditions (Lindstrom and Mousavi, 2019; Sinharoy and Tian, 2024). Collectively, these seven isolates form a strong foundation for developing nitrogen-fixing biofertilizers in hydroponic system.

Phosphate solubilizing ability on Pikovskaya medium indicated by clear zone formation was detected in five isolates (M15, M13, J12, N21, L13), reflecting organic acid secretion as the primary mechanism. This uneven functional distribution aligns with literature showing that P solubilization depends on genetic capacity for organic acid and phosphatase production, microenvironmental pH and mineral composition, and carbon availability from root exudates (Pan and Cai, 2023; Sanchez-gonzalez et al., 2023; Santos et al., 2025). Quantitative assays again identified M13 as the strongest performer, consistent with multifunctional isolates capable of expressing multiple metabolic pathways simultaneously, while L13 and J12 contributed lower but stable activity.

Potassium solubilization was detected in six isolates based on clear zones on Alexandrov medium, with differences in zone diameter reflecting metabolic heterogeneity (Adwani et al., 2024). K solubilization is controlled by organic-acid biosynthesis and strain-specific genetic factors (Babar et al., 2024). Quantitative data identified A34 as the most effective K solubilizer, followed by M13, which again exhibited a broad functional spectrum commonly described as integrated nutritional capability (Azizah et al., 2020). Isolates with lower activity such as E12 remain relevant for gradual K mobilization.

IAA production was detected in six isolates (A34, B31, D12, G31, K31, M13), with color variation in the Salkowski test indicating differences in biosynthetic regulation influenced by L-tryptophan availability, aminotransferase activity, and local physicochemical conditions (Ratnaningsih et al., 2023; Zhang et al., 2021). Quantitative tests showed A34 as the highest IAA producer, and its combined ability to solubilize K and synthesize IAA highlights its potential as a dual-function biofertilizer candidate for nutrient mobilization and growth promotion.

Biofilm analysis exhibited distinct dynamics from nutritional traits, as only two isolates (J31 and J12) formed detectable structures, and under consortium testing only J31 maintained stable biofilm formation (Figure 6). This demonstrates that biofilm capability is highly selective and shaped by isolate physiology and inter-microbial interactions (Yao et al., 2022). Biofilm formation requires complex regulation including EPS production, quorum sensing, and cell surface architecture (Wang et al., 2023) which may explain why only one isolate consistently retained this ability in both solo and consortium conditions. The inability of other isolates to maintain biofilms suggests competitive interactions that suppress matrix formation.

Overall, the results of this study show that the rhizosphere bacterial community of *L. flava* in substrate based hydroponic systems has a diverse

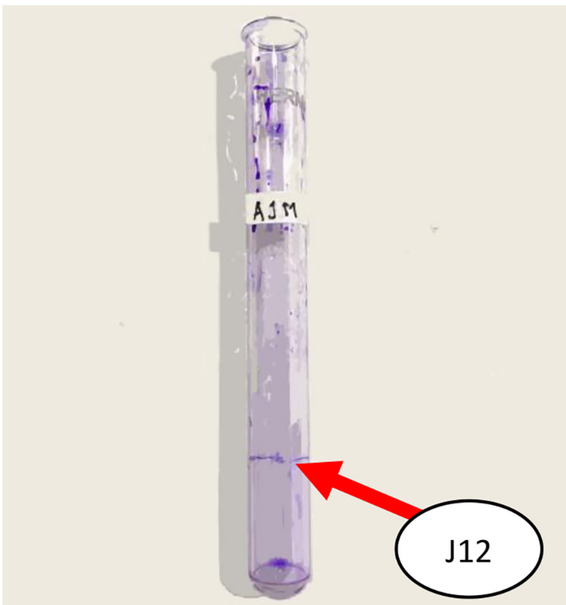

Figure 6. Identified bacterial isolates have the ability to form biofilms in consortium conditions

range of ecological functions, from N fixation, P and K mobilization, IAA biosynthesis, to biofilm formation. The dominance of several isolates such as A34, J31, and M13 indicates the presence of key candidates that can be further developed into multifunctional biofilm biofertilizers (Table 7). This diversity of functions confirms that the root microbiome in hydroponics is not homogeneous, but rather composed of groups with complementary ecological contributions.

The combination of superior characteristics in isolates A34, J31, and M13 indicates a very promising consortium configuration for the development of biofilm biofertilizers. Isolate A34, which has a high capacity for potassium solubilization and IAA production, is consistent with findings that K-solubilizing bacteria and phytohormone-producing bacteria can enhance root expansion and nutrient uptake efficiency in modern cropping systems (Singhmar et al., 2024). Isolate J31, as the isolate with the most stable biofilm formation ability, plays an important role as a supporting structure that allows the consortium to survive in the root zone, in line with the concept that the biofilm matrix enhances cell protection, nutrient retention, and microbial colonization effectiveness (Bhattacharyya et al., 2023). On the other hand, M13, which is capable of nitrogen fixation and phosphate solubilization, provides bioavailable N and P supplies, supporting plant vegetative growth as reported in diazotrophs and P-solubilizing bacteria used in precision agriculture systems (Martinez-feria et al., 2024; Ríos-Ruiz et al., 2024). When synergized, these three characters produce a consortium of bacteria with N fixation, P and K solubilization, IAA production, and biofilm formation functions that are highly compatible with the latest approach in the

Table 7. Summary of the ability of 23 bacterial isolates to fix N, solubilize P and K, produce IAA, and form biofilms

Isolat code	Bacterial potential					Selected isolates	Notes
	Fixing N	Solubizing P	Solubizing K	Producing IAA	Forming biofilm		
A34	-	-	++	++	-	A34	Excellent at dissolving K and producing IAA
B22	+	-	+	-	-		
B31	-	-	-	+	-		
B34	-	-	-	-	-		
C13	-	-	-	-	-		
C24	+	-	-	-	-		
D12	-	-	+	+	-		
E12	-	-	+	-	-		
F21	-	-	-	-	-		
G14	-	-	-	-	-		
G22	-	-	-	-	-		
G31	+	-	-	+	-		
H11	-	-	-	-	-		
I31	+	-	-	-	-		
J12	-	+	-	-	+		
J31	-	-	-	-	++	J31	Forming biofilms in single and consortium conditions
K31	+	-	-	+	-		
L13	-	+	+	-	-		
M13	++	++	+	+	-	M13	Excellent at fixing N and dissolving P
M15	-	+	-	-	-		
N11	-	-	-	-	-		
N21	-	+	-	-	-		
O21	+	-	-	-	-		

Notes: (+) indicates a positive activity, (++) indicates strong or high activity, and (–) denotes no detectable activity.

development of multifunctional microbe-based biofertilizers. These three isolates are recommended as the main candidates in the formulation of effective biofilm biofertilizers to improve plant performance in sustainable hydroponic systems. Nevertheless, functional screening was conducted under in vitro conditions, and further studies are required to validate the performance of selected isolates under in vivo hydroponic cultivation and in microbial consortia.

CONCLUSIONS

In conclusion, this study successfully isolated and selected rhizosphere bacteria associated with *L. flava* in a hydroponic system. The diversity of morphological and physiological characteristics identified indicates that the rhizosphere of *L. flava* is a habitat for bacterial communities with functional potential relevant to the development of biofertilizer biofilms. Functional tests revealed several superior isolates with the ability to fix nitrogen, solubilize phosphate, produce IAA, and form stable biofilms in consortium conditions. Three isolates, namely A34 (K solubilizer and IAA producer), J31 (stable biofilm former), and M13 (N fixer and P solubilizer) emerged as the most promising candidates. These findings confirm that the rhizosphere of *L. flava* provides a source of adaptive local microbes that have the potential to be utilized for biofertilizer formulations suitable for hydroponic conditions. However, further verification through application tests on a field hydroponic scale is needed to ensure the consistency of performance and feasibility of utilizing these isolates in sustainable hydroponic cultivation systems.

Acknowledgements

This research received support from *Pusat Pembiayaan dan Asesmen Pendidikan Tinggi* (PPAPT, or Center for Higher Education Funding and Assesment) and *Lembaga Pengelolaan Dana Pendidikan* (LPDP, or the Indonesia Endowment Funds for Education) through *Beasiswa Pendidikan Indonesia* (BPI, or the Indonesia Education Scholarship) of the Ministry of Higher Education, Science, and Technology of Republik Indonesia (*Kementerian Pendidikan Tinggi, Sains, dan Teknologi Republik Indonesia*) with the scholarship recipients identification number 202209092021.

REFERENCES

1. Adwani, M. S., Pillai, T. S., Isokar, S. S., Rokade, G. S. (2024). Evaluation and characterization of potassium solubilizing bacteria. *International Journal of Advanced Biochemistry Research*, *8*(5), 245–248. https://doi.org/10.33545/26174693.2024.v8.i5Sd.1174
2. Aliyat, F. Z., Maldani, M., Guilli, M. El, Nassiri, L., Ibijbijen, J. (2022). Phosphate-solubilizing bacteria isolated from phosphate solid sludge and their ability to solubilize three inorganic phosphate forms: calcium, iron, and *Aluminum phosphates*. *Microorganisms*, *10*(980), 1–13. https://doi.org/10.3390/microorganisms10050980
3. Alkaabi, A., Almansoori, E., Hebsi, M. A. L., Aldhaheri, S., Hassan, F. E., Ali, N. A. A., Shurafa, K. A. Al, Tzortzakis, N., Gioia, F. Di, Ahmed, Z. F. R. (2025). Vertical hydroponic lettuce: impact of organic nutrients on antioxidant phytochemicals. *Annals of Agricultural Sciences*, *70*(100386), 1–11. https://doi.org/10.1016/j.aoas.2025.100386
4. Amalina, Sudadi, Pratama, N., Dewi. (2023). The potency of soil bacteria on soil phosphorus solubilization and IAA production for innovation on sustainable Melon (*Cucumis melo*) production through biofilm biofertilizer. *IOP Conference Series: Earth and Environmental Science*, 1–8. https://doi.org/10.1088/1755-1315/1200/1/012001
5. Azizah, H., Rahajeng, S. M., Jatmiko, Y. D. (2020). Isolation and screening of phosphate and potassium solubilizing endophytic bacteria in maize (*Zea mays* L.). *J.Exp. Life Sci*, *10*(3), 165–170.
6. Babar, S., Baloch, A., Qasim, M., Wang, J., Wang, X. (2024). Unearthing the soil-bacteria nexus to enhance potassium bioavailability for global sustainable agriculture: A mechanistic preview. *Microbiological Research*, *288*(127885). https://doi.org/10.1016/j.micres.2024.127885
7. Banboukian, A., Chen, Y., Thomas, V. M. (2025). The challenges of controlled environment hydroponic farming : a life cycle assessment of lettuce. *The International Journal of Life Cycle Assessment*, *30*, 1691–1704. https://doi.org/https://doi.org/10.1007/s11367-025-02463-6
8. Basnet, A., Tamang, B., Shrestha, M. R., Shrestha, L. B., Rai, J. R., Maharjan, R., Dahal, S., Shrestha, P., Rai, S. K. (2023). Assessment of four in vitro phenotypic biofilm detection methods in relation to antimicrobial resistance in aerobic clinical bacterial isolates. *Plos One*, *18*(11), 1–15. https://doi.org/10.5061/dryad.wh70rxwtg
9. Bennett, E. M., Murray, J. W., Isalan, M. (2023). Engineering nitrogenases for synthetic nitrogen fixation : from pathway engineering to directed evolution. *Bio Design Research*, *5*(5), 1–12. https://doi.org/10.34133/bdr.0005

10. Bhattacharyya, A., Mavrodi, O., Bhowmik, N., Weller, D., Mavrodi, D., Sciences, E., States, U., Health, W., States, U. (2023). Bacterial biofilms as an essential component of rhizosphere plant-microbe interactions. *Methods Microbiol*, *53*(3). https://doi.org/10.1016/bs.mim.2023.05.006
11. Brokate, O., Papenbrock, J., Turcios, A. E. (2024). Biofilm-forming microorganisms in the rhizosphere to improve plant growth : Coping with abiotic stress and environmental pollution. *Applied Soil Ecology*, *202*(105591), 1–17. https://doi.org/10.1016/j.apsoil.2024.105591
12. Chhetri, S., Sherpa, M. T., Sharma, L. (2025). Characterization of plant growth promoting bacteria isolated from rhizosphere of tomato cultivated in Sikkim Himalaya and their potential use as biofertilizer. *Scientific Reports*, *15*(1), 1–16. https://doi.org/10.1038/s41598-025-98953-6
13. Chowdhury, M., Samarakoon, U. C. (2024). Evaluation of hydroponic systems for organic lettuce production in controlled environment. *Plant Science*, *15*(1401089), 1–12. https://doi.org/10.3389/fpls.2024.1401089
14. Crivelli, X. B., Cundon, C., Sanin, M. S., Bentancor, A. (2024). The complex and changing *Genus Bacillus*: A diverse bacterial powerhouse for many applications. *Bacteria*, *3*, 256–270. https://doi.org/10.3390/ bacteria3030017
15. Gangthilaka, K. M. S. M., Premarathna, M., Madawala, H. M. S. P., Senviratne, G. (2022). Can biofilm biofertilizer cut down chemical fertilizers in leafy vegetable cultivation ? A case study with *Centella asiatica* (Gotukola). *The Journal of Agricultural Sciences -*, *17*(3), 370–378. https://doi.org/10.4038/jas.v17i3.9918
16. Gen-jiménez, A., Flores-félix, J. D., Rincón-molina, C. I., Manzano-gomez, L. A., Rogel, M. A., Ruíz-valdiviezo, V. M., Rincón-molina, F. A. (2023). Enhance of tomato production and induction of changes on the organic profile mediated by *Rhizobium biofortification*. *Front. Microbiol*, *14*(1235930), 1–13. https://doi.org/10.3389/fmicb.2023.1235930
17. Gerrewey, T. Van, El-nakhel, C., Pascale, S. De, Paepe, J. De, Clauwaert, P., Kerckhof, F., Boon, N., Geelen, D. (2021). Root-associated bacterial community shifts in hydroponic lettuce cultured with urine-derived fertilizer. *Microorganisms*, *9*(1326), 1–25. https://doi.org/10.3390/microorganisms9061326
18. Goa, T., Beyene, G., Mekonnen, M., Gorems, K. (2022). Isolation and characterization of lactic acid bacteria from fermented milk produced in Jimma Town, Southwest Ethiopia, and evaluation of their antimicrobial activity against selected pathogenic bacteria. *International Journal of Food Science*, *15*(2076021), 1–15. https://doi.org/10.1155/2022/2076021
19. Goulart, J. T. de S. S., Quintanilha-Peixoto, G., Esteves, B. dos S., Souza, S. A. de, Lopes, P. S., Silva, N. D. da, Soares, J. R., Barroso, L. M., Suzuki, M. S., Intorne, A. C. (2024). Isolation and characterization of plant-growth-promoting bacteria associated with *Salvinia auriculata* Aublet. *Microorganisms*, *12*(1842), 1–13. https://doi.org/10.3390/microorganisms12091842
20. Guevara, F., Frey, T., Malacrinò, A., Soledad, M., Ponce, B. (2025). Influence of system type and management practices on bacterial and fungal community structure in hydroponic systems: Insights from commercial facilities in Ohio. *Phytobiomes Journal*, *9*(2), 302–316. https://doi.org/10.1094/PBIOMES-07-24-0070-R
21. Hardiansyah, M. Y., Musa, Y., Jaya, A. M. (2020). Identification of plant growth promoting rhizobacteria from thorny bamboo rhizosphere with 3% KOH gram test and gram staining test. *International Journal of Applied Biology*, *4*(2), 7–17.
22. Itkina, D., Pudova, D., Drozdova, M., Sharipova, M. (2025). Genetic potential of *Bacillus ginsengihum*i as a basis for a combined biofertilizer. *BIO Web of Conferences*, *173*(03036), 1–12. https://doi.org/10.1051/bioconf/202517303036
23. Kalniņš, M., Andersone-Ozola, U., Gudrā, D., Alise Sieriņa, Fridmanis, D., Ievinsh, G., Muter, O. (2022). Effect of bioaugmentation on the growth and rhizosphere microbiome assembly of hydroponic cultures of *Mentha aquatica*. *Ecological Genetics and Genomics Effect*, *22*(100107), 1–9. https://doi.org/10.1016/j.egg.2021.100107
24. Kaundal, S., Rana, N., Kumar, Y., Alhewairini, S. S., Barasarathi, J. (2025). Bio fi lmed multifarious rhizobacterial isolates of tomato rhizosphere of North-Western Himalayas promote plant growth in tomato. *Frontiers in Plant Science*, *16*(1610707), 1–14. https://doi.org/10.3389/fpls.2025.1610707
25. Khatoon, H., Chavan, D. D., Anokhe, A., Kalia, V. (2022). Catalase test: A biochemical protocol for bacterial identificatio. *Agricos E-Newsletter*, *3*(1), 53–55.
26. Lam, N. T., Song, S., Thi, B., Dung, N., Binh, T. N., Maleki, A. (2022). Potential role of combined microbial inoculants and plant of limnocharis flava on eliminating cadmium from artificial contaminated soil. *Sustainability*, *14*(12209), 1–14. https://doi.org/10.3390/su141912209
27. Lebrazi, S., Fadil, M., Chraibi, M., Fikri-benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production by *Rhizobium* sp. strain using response surface methodology. *Journal of Genetic Engineering and Biotechnology*, *18*(21), 1–10. https://doi.org/10.1186/s43141-020-00035-9
28. Li, Y., Yang, R., Khan, A., Soleimani, M., Guo, L., Sun, X., Li, B., Liu, H., Jin, S., Sun, W. (2024).

Keystone rhizospheric diazotrophs may promote the pioneer plant growth in mine tailings. *Soil Security*, *16*(100140), 1–10. https://doi.org/10.1016/j.soisec.2024.100140

29. Linda, T. M., Febriarti, B. L., Zul, D., Sofyanti, N., Berliansyah, A., Delfira, N., Devi, S. (2023). Isolation and characterization of endophytic bacteria from sterile leaf of *Acrostichum aureum* from Bengkalis Island (Riau, Indonesia) and its potency for antidiabetic. *Biodiversitas*, *24*(3), 1580–1588. https://doi.org/10.13057/biodiv/d240330
30. Lindstrom, K., Mousavi, S. A. (2019). Minireview Effectiveness of nitrogen fi xation in rhizobia. *Microbial Biotechnology*, *0*(0), 1–22. https://doi.org/10.1111/1751-7915.13517
31. Makino, A., Nakai, R., Yoneda, Y., Toyama, T., Tanaka, Y., Meng, X. (2022). Isolation of aquatic plant growth-promoting bacteria for the floating plant duckweed (*Lemna minor*). *Microorganisms*, *10*(1564), 1–18. https://doi.org/10.3390/microorganisms10081564
32. Martínez-Cámara, R., Montejano-Ramírez, V., Moreno-Hagelsieb, G., Santoyo, G., Valencia-Cantero, E. (2020). The volatile organic compound dimethylhexadecylamine affects bacterial growth and swarming motility of bacteria. *Folia Microbiologica*, *65*(3), 523–532. https://doi.org/10.1007/s12223-019-00756-6
33. Martinez-feria, R., Simmonds, M. B., Ozaydin, B., Lewis, S., Schwartz, A., Pluchino, A., Mckellar, M., Gottlieb, S. S., Kayatsky, T., Vital, R., Mehlman, S. E., Caron, Z., Colaianni, N. R., Ané, J., Maeda, J., Infante, V., Karlsson, B. H., Mclimans, C., Vyn, T.,… Tamsir, A. (2024). Genetic remodeling of soil diazotrophs enables partial replacement of synthetic nitrogen fertilizer with biological nitrogen fixation in maize. *Scientific Reports*, *14*(27754), 1–18. https://doi.org/10.1038/s41598-024-78243-3 1
34. Masi, C., Gemechu, G., Tafesse, M. (2021). identification of alkaline protease- producing bacteria from leather industry effluent. *Annals of Microbiology*, *71*(24), 1–11. https://doi.org/10.1186/s13213-021-01631-x
35. Miller, J. H., Fasanello, V. J., Liu, P., Longan, E. R., Botero, C. A., Id, J. C. F. (2022). Using colony size to measure fitness in *Saccharomyces cerevisiae*. *Plos One*, *17*(10), 1–18. https://doi.org/10.1371/journal.pone.0271709
36. Mondal, S., Biswas, B., Chowdhury, R., Sengupta, R., Mandal, A., Kotal, H. N., Giri, C. K., Ghosh, A., Saha, S., Begam, M. M., Mukherjee, C., Das, I., Basak, S. K., Ghosh, M. M., Krishna, R. (2024). Estuarine mangrove niches select cultivable heterotrophic diazotrophs with diverse metabolic potentials – a prospective cross-dialog for functional diazotrophy. *Front. Microbiol*, *15*(1324188), 1–21. https://doi.org/10.3389/fmicb.2024.1324188
37. Nguyen, N. K., Vo, D. T. V., Le, T. X., Morton, L. W., Tran, H.-T., Robatjazi, J., Lasar, H. G. W., Tecimen, H. B. (2024). Biocatalysis and agricultural biotechnology isolation, and selection of indigenous potassium solubilizing bacteria from Vietnam Mekong Delta rhizospheric soils and their effects on diverse cropping systems. *Biocatalysis and Agricultural Biotechnology*, *58*(103200), 1–15. https://doi.org/10.1016/j.bcab.2024.103200
38. Oktari, I., Mustamu, N. E., Walidav, H. (2021). Ultisols fertility and morphological characteristics of N-fixing bacteria from oil palm rhizosphere. *Pesquisa Agropecuaria Tropical*, *51*(68559), 1–7. https://doi.org/10.1590/1983-40632021v5168559
39. Orlandi, V. T., Martegani, E., Giaroni, C., Baj, A., Bolognese, F. (2022). Bacterial pigments: A Colorful palette reservoir for biotechnologycal applications. *Wiley*, *69*, 981–1001. https://doi.org/10.1002/bab.2170
40. Pan, L., Cai, B. (2023). Phosphate-solubilizing bacteria: Advances in their physiology, molecular mechanisms and microbial community effects. *Microorganisms*, *11*(2904), 1–22. https://doi.org/10.3390/microorganisms11122904
41. Premarathna, M., Seneviratne, G., Ketipearachchi, K. G., Science, R., Balasooriya, W. K. (2021). Biofilm biofertilizer can reinstate network interactions for improved rice production. *Ceylon Journal of Science*, *50*(3), 235–242. https://doi.org/10.4038/cjs.v50i3.7904
42. Pujiati, Prasetyanti, Y. W., Ardhi, M. W., Fatimah, Ramadhan, R., Ni'matuzahroh. (2025). Isolation and potential test of indigenous bacteria from Singolangu, Magetan, Indonesia for bioremediation of organophosphate compounds. *Biodiversitas*, *16*(3), 1029–1038. https://doi.org/10.13057/biodiv/d260301
43. Putra, S. P., Carolina, Y., Salsinha, F. (2023). Waterlogging and salinity stress affecting growth and morphological character changes of Limnocharis flava. *Biodiveritas*, *24*(1), 333–340. https://doi.org/10.13057/biodiv/d240140
44. Rafique, M., Naveed, M., Mumtaz, M. Z., Niaz, A., Alamri, S., Siddiqui, M. H., Waheed, M. Q., Ali, Z., Naman, A., Rehman, S., Brtnicky, M., Mustafa, A. (2024). Unlocking the potential of biofilm - forming plant growth - promoting rhizobacteria for growth and yield enhancement in wheat (*Triticum aestivum* L.). *Scientific Reports*, *14*(15546), 1–18. https://doi.org/10.1038/s41598-024-66562-4
45. Ratnaningsih, H. R., Noviana, Z., Kumala, T., Loekito, S. (2023). Heliyon IAA and ACC deaminase producing-bacteria isolated from the rhizosphere of pineapple plants grown under different abiotic and biotic stresses. *Heliyon*, *9*(16306), 1–13. https://doi.org/10.1016/j.heliyon.2023.e16306

46. Ríos-Ruiz, W. F., Tarrillo-Chujutalli, R. E., Rojas-García, J. C., Tuanama-Reátegui, C., Pompa-Vásquez, D. F., Zumaeta-Arévalo, C. A. (2024). The biotechnological potential of plant growth-promoting rhizobacteria isolated from maize (*Zea mays* l.) cultivations in the San Martin Region, Peru. *Plants*, *13*(2075), 1–19. https://doi.org/10.3390/plants13152075
47. Salo, E. N., Novero, A. (2020). Identification and characterisation of endophytic bacteria from coconut (*Cocos nucifera*) Tissue Culture Authors: Elv Nhiel Salo and Annabelle Novero * * Correspondence: aunovero@up.edu.ph https://doi.org/10.21315/tlsr2020.31.1.4 Highlights • • F. *Tropical Life Sciences Research*, *31*(1), 57–68. https://doi.org/10.21315/tlsr2020.31.1.4
48. Samet, M., Ghazala, I., Karray, F., Abid, C., Chiab, N., Nouri, O. (2022). Isolation of bacterial strains from compost teas and screening of their PGPR properties on potato plants. *Environmental Science and Pollution Research*, *29*, 75365–75379. https://doi.org/10.1007/s11356-022-21046-8
49. Sanchez-gonzalez, M. E., Mora-herrera, M. E., Wong-villarreal, A., La, N. De, Sanchez-paz, L., Lugo, J., Vaca-paul, R., Aguila, P. Del, Yañez-ocampo, G. (2023). Effect of pH and carbon source on phosphate solubilization by bacterial strains in Pikovskaya Medium. *Microorganisms*, *11*(49), 1–11. https://doi.org/10.3390/microorganisms11010049
50. Santos, J., Cardoso, P., Rocha, R., Pinto, R., Lopes, T., Patinha, C., Guilherme, R., Ferreira, A., Figueira, E. (2025). Phosphate-Solubilizing bacteria from different genera, host plants, and climates: Influence of soil pH on plant growth and biochemistry. *Land*, *14*(2065), 1–24. https://doi.org/10.3390/land14102065
51. Sarti, G. C., Galelli, M. E., Ana, J., Crist, E., Eliana, C., Chudil, H. D., Rosa, A., Paz-gonz, A. (2024). Inoculation with biofilm of bacillus subtilis Is a safe and sustainable alternative to promote tomato (*Solanum lycopersicum*) growth. *Environments*, *11*(54), 1–18. https://doi.org/10.3390/ environments11030054
52. Schmautz, Z., Espinal, C. A., Bohny, A. M., Rezzonico, F., Junge, R., Frossard, E., Smits, T. H. M. (2021). Environmental parameters and microbial community profiles as indication towards microbial activities and diversity in aquaponic system compartments. *BMC Microbiology*, *21*(12), 1–11. https://doi.org/10.1186/s12866-020-02075-0
53. Sheikh, J., Swee, T. T., Malik, S., Saidin, S., Chua, L. S. (2024). Bacterial morphology and microscopic advancements: navigating from basics to breakthroughs. *Microbial and Immunological Communications*, *3*(1), 3–41. https://doi.org/10.55627/mic.003.01.0567
54. Shi, P., Zhang, J., Li, X., Zhou, L., Luo, H., Wang, L., Zhang, Y., Chou, M., Wei, G. (2022). Multiple metabolic phenotypes as screening criteria are correlated with the plant growth-promoting ability of *Rhizobacterial Isolates*. *Front. Microbiol*, *12*(747982), 1–16. https://doi.org/10.3389/fmicb.2021.747982
55. Shi, Z., Guo, X., Lei, Z., Wang, Y., Yang, Z., Niu, J., Liang, J. (2023). Screening of high - efficiency nitrogen - fixing bacteria from the traditional Chinese medicine plant *Astragalus mongolicus* and its effect on plant growth promotion and bacterial communities in the rhizosphere. *BMC Microbiology*, *23*(292), 1–18. https://doi.org/10.1186/s12866-023-03026-1
56. Singhmar, R., Singh, V., Bhatti, L., Dalal, S., Malik, D. K. (2024). Inoculation of multi-trait plant growthpromoting rhizobacteria for wheat growth promotion in pothouse conditions. *Journal of Microbiol Biotech Food Sciences*, *14*(3), 1–6. https://doi.org/10.55251/jmbfs.10484
57. Sinharoy, S., Tian, C. (2024). Editorial : Plant-rhizobia symbiosis and nitrogen fi xation in legumes. *Front. Plant Sci*, *15*(1392006), 2022–2024. https://doi.org/10.3389/fpls.2024.1392006
58. Sousa, A. M., Machado, I., Nicolau, A., Pereira, M. O. (2013). Improvements on colony morphology identi fi cation towards bacterial pro fi ling. *Journal OfMicrobiological Methods*, *95*, 327–335. https://doi.org/10.1016/j.mimet.2013.09.020
59. Spencer, L., Costine, B., Irwin, T., Dixit, A., Spern, C., Diaz, A., Lozzi, B., Li, W., Khodadad, C., Smith, T., Wheeler, R., Rourke, A. O. (2024). Substrate matters: ionic silver alters lettuce growth, nutrient uptake, and root microbiome in a hydroponics system. *Microorganisms*, *12*(515). https://doi.org/10.3390/microorganisms12030515
60. Stegelmeier, A. A., Rose, D. M., Joris, B. R. (2022). The use of PGPB to promote plant hydroponic growth. *Plants*, *11*(2783), 1–28. https://doi.org/10.3390/plants11202783
61. Sudewi, S., Ala, A., Baharuddin, Farid, M. (2020). The isolation, characterization endophytic bacteria from roots of local rice plant kamba in, central sulawesi, indonesia. *Biodiversitas*, *21*(4), 1614–1624. https://doi.org/10.13057/biodiv/d210442
62. Sun, D., Zhang, H., Pan, G., Zhang, Z., Xing, J., Li, J., Gao, Y. (2025). Impact of tetracycline stress on water quality and rhizosphere microbial communities of Eichhornia crassipes: Implications for bioremediation. *Microorganisms*, *13*(893). https://doi.org/10.3390/microorganisms13040893
63. Wagh, J., Bhandari, P., Shah, S. (2014). Overexpression of citrate operon in *Herbaspirillum seropedicae* Z67 enhances organic acid secretion, mineral phosphate solubilization and growth promotion of Oryza sativa. *Palnt Soil*, *383*, 73–86. https://doi.org/10.1007/s11104-014-2161-2

64. Wang, C., Wang, H., Li, Y., Li, Q., Yan, W., Zhang, Y. (2021). Plant growth-promoting rhizobacteria isolation from rhizosphere of submerged macrophytes and their growth-promoting effect on Vallisneria natans under high sediment organic matter load. *Microbial Biotechnology*, *14*(2), 726–736. https://doi.org/10.1111/1751-7915.13756
65. Wang, Xinyu, Liu, M., Yu, C., Li, J., Zhou, X. (2023). Biofilm formation: mechanistic insights and therapeutic targets. *Molecular Biomedicine*, *4*(49), 1–25. https://doi.org/10.1186/s43556-023-00164-w
66. Yao, S., Wu, C., Hao, L. (2022). Multispecies biofilms in fermentation: Biofilm formation, microbial interactions, and communication. *Compreh Ensive Reviews In Food Science And Food Safety*, *21*(3346), 3346–3375. https://doi.org/10.1111/1541-4337.12991
67. Zhang, B.-X., Li, P., Wang, Y., Wang, J., Liu, X., Wang, X., Hu, X. (2021). acid in plant growth promoting *Enterobacter* sp. †. *Royal Society of Chemistry*, *11*(31601), 31601–31607. https://doi.org/10.1039/d1ra05659j