

Synergistic effects of nitrogen-fixing bacteria and varying coir dust ratios on plant growth: A green approach

Jayathilake K.M.P.I.^{1,2}, Manage P.M.¹, Idroos F. S.¹✉

¹Centre for Water Quality and Algae Research, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka.

²Faculty of Graduate Studies, University of Sri Jayewardenepura, Sri Lanka.

✉ Corresponding author: sumaiyaidroos@sci.sjp.ac.lk

Article info

Article history:

Received: 13th February 2025

Accepted: 24th March 2026

Keywords:

Nitrogen-fixing bacteria
Biofertilizer
Plant Growth Promotion
Bacillus spp.
Sustainable Agriculture

Abstract

Nitrogen-fixing bacteria (NFB) play a vital role in sustainable agriculture. They enhance soil nitrogen availability, reduce reliance on synthetic fertilizers, and promote plant growth. This study aimed to isolate and characterize NFB strains from compost soil and evaluate their potential to improve nitrogen uptake and plant growth. Genotypic identification confirmed that the isolated strains belonged to six species of the *Bacillus* genus and one *Microbacterium aquimaris* species. All isolated *Bacillus* spp. strains and *Microbacterium aquimaris* tested positive for nitrogen-fixing activity and ammonia production tests. The highest ammonia (1.6 ± 0.5 mg/L) and nitrate (1.08 ± 0.04 mg/L) production were recorded in Ashby broth medium inoculated with the *Bacillus cereus* strain after 7 days of incubation period. The potting media consisted of a ratio 1 inoculated with the *Bacillus cereus* strain CCM 2010, which exhibited the highest growth parameters, including maximum root length (11.3 ± 1.1 cm), shoot length (17.5 ± 0.5 cm), wet weight (0.6898 ± 0.1 g), and dry weight (0.1015 ± 0.01 g). These findings suggest that integrating NFB with varying ratios of coir dust in potting media significantly enhances plant growth, suggesting a viable and sustainable approach to improving agricultural productivity.

© University of SS. Cyril and Methodius in Trnava

Introduction

Nitrogen-fixing bacteria (NFB) improve soil fertility and contribute to sustainable agriculture by converting atmospheric nitrogen into a form accessible to plants. This natural process reduces the dependency on synthetic fertilizers, which are both costly and environmentally harmful (Vats *et al.* 2021; Monib *et al.* 2024; Jayathilake *et al.*, 2025; Jayathilake *et al.*, 2026). In recent years, the isolation and characterization of these bacteria from various soil types have gained significant attention. Compost soil enriched with organic matter provides

a conducive environment for a diverse microbial population (Bamdad *et al.* 2022). Understanding the specific bacteria involved in nitrogen fixation can enhance soil fertility management. Microbial inoculants such as NFB have proven effective in enhancing soil fertility and crop yields by improving nutrient availability. Compost soil, with its diverse microbial population, is ideal for sourcing these beneficial microbes (Sammauria *et al.* 2020). Characterizing NFB involves assessing nitrogen fixation rates, plant growth-promoting

properties, and compatibility with soil amendments like coir dust (Kumar *et al.* 2023; Sharma *et al.* 2023).

Coir dust, a byproduct of coconut processing, is increasingly recognized for its potential to improve soil structure and fertility. When used as a soil amendment, coir dust can enhance water retention, aeration, and microbial activity, creating a favorable environment for plant growth (Atzori *et al.* 2021; Sivaram *et al.* 2023). Incorporating coir dust into agricultural practices aligns with sustainable and eco-friendly farming approaches (Chromkaew *et al.* 2023; Sivaram *et al.* 2023). However, the optimal ratio of coir dust to soil for maximizing plant growth and health remains underexplored. Combining NFB with coir dust amendments may create a synergistic effect that promotes plant growth and soil health. The NFB benefits from the improved soil structure and moisture retention of coir dust, potentially enhancing their activity and nitrogen availability to plants. This interaction represents an innovative approach to integrating microbial inoculants and organic soil amendments (Sivaram *et al.* 2023).

The objective of this research is to isolate and characterize NFB from compost soil to harness their potential benefits. It also investigates the effect of varying coir dust ratios on plant growth, aiming to identify the most beneficial proportions. This research explores the combined impact of NFB and coir dust on plant growth to develop a comprehensive strategy for sustainable agriculture.

Experimental

Soil sample collection

The soil sample was collected from compost sites in Karadiyana (6°48' 51.8" N, 79° 54' 17.0" E) for the isolation process of NFB. Each sample was kept in clean, sterile bags, sealed, and transferred to the laboratory.

Analysis of physicochemical parameters of soil samples

Soil pH, Electrical Conductivity (EC), total soil carbon and soil organic nitrogen were determined following standard protocols and methods.

(Walkley and Black 1934; AOAC 2000; Mussa *et al.* 2009).

Isolation of NFB

NFB strains were isolated using Ashby medium agar. A series of 10-fold dilutions of the suspension was prepared for each sample. 100µl of each concentration was spread directly onto the surface of Ashby medium plates. Plates were incubated at 30°C for 3 days. The NFB were identified using colony characteristics. Isolated bacterial strains were labeled as NFB-1, NFB-2, NFB-3, NFB-4, NFB-5, NFB-6 and NFB-7.

Biochemical analysis of bacterial strains

Rhizospheric microbes were characterized by different biochemical methods, Gram Reaction, Motility Test, Spore Staining, Catalase Test, Citrate Utilization Test, Starch Hydrolysis Test and Spot Test for Assessment of Indole Production (Hemraj *et al.* 2013; Hamza *et al.* 2017; AL-Joda and Jasim 2021).

Gram staining

A thin smear of the isolated pure culture strains was prepared on a glass slide. The smear was air-dried on a hot plate and then heat-fixed using a Bunsen burner for 3-5 seconds. The smear was covered with Crystal violet for 60 seconds and then briefly rinsed with water. Subsequently, the smear was covered with Gram's iodine for 60 seconds and rinsed thoroughly with water. The smear was then covered with 95% ethanol for 10 seconds and immediately washed with water. Safranin was applied to the smear for 60 seconds, followed by a thorough rinse with water. The slide was carefully blotted with a paper towel. A small drop of immersion oil was applied to the smear, then examined under a high-power microscope (100x objective) (Hamza *et al.* 2017).

Motility test

This procedure was performed to determine the presence of locomotor structures. A drop of cultured organisms suspended in saline was placed

on a slide and covered with a coverslip. The slide was then examined under a microscope. Observing sharp, darting movements in various directions across the field of view indicated positive motility, suggesting the presence of locomotive structures (Hamza et al. 2017).

Spore staining

This procedure was carried out to determine whether the test organisms were spore producers. A heat-fixed smear of the strains was subjected to boiling using a mixture of 5% aqueous malachite green and 0.5% safranin. A heat-fixed smear of the sample was made on clean slides. The slide was flooded with malachite green and heated from below with the Bunsen flame. When the flame was removed, steam was seen rising from the stain. The dye was allowed to act with the steam rising from it for a minute. The stain was washed off thoroughly with distilled water, and the slides were drained. The slides were also flooded with safranin and left to act for 30 seconds. The slides were washed off with distilled water, drained and blotted dry with absorbent paper. When completely dried, a drop of immersion oil was added and observed under the microscope, showing the spores' pale green colour if the isolate was a spore producer (Hamza et al. 2017).

Catalase test

This test examined the presence of the catalase enzyme in the tested organism. Catalase activity was examined by suspending one loopful of the organism in a drop of 3% hydrogen peroxide on a glass slide. This test was performed as per standard procedure. The production of bubbles indicates a positive result for the catalase test (Hamza et al. 2017; AL-Joda and Jasim 2021).

Citrate Utilization Test

This test was conducted to determine whether the test organism could utilize citrate as a carbon source. The organism was incubated on Simmon's citrate agar slants, which contained the following composition: ammonium dihydrogen phosphate (1.0 g/L), dipotassium hydrogen phosphate (1.0

g/L), sodium chloride (5.0 g/L), sodium citrate (2.0 g/L), magnesium sulfate (0.2 g/L), bromothymol blue (0.08 g/L), and agar-agar (13.0 g/L). A distinct colour change from green to blue indicated a positive result for citrate utilization (AL-Joda and Jasim 2021).

Starch Hydrolysis Test

This test was conducted to confirm the test organism's production of amylase enzymes. The organism was incubated on a nutrient agar plate containing 1% starch for 48 hours. Following incubation, a drop of iodine solution was added to the plate. A clear zone around the colony indicated a positive result for starch hydrolysis (Hemraj et al. 2013; AL-Joda and Jasim 2021).

Spot Test for Assessment of Indole Production

Spot test for indole production was performed by growing selected strains on Tryptone Soya Agar medium amended by adding glucose (10 g); K_2HPO_4 (0.5 g); $MgSO_4 \cdot 7H_2O$ (0.2 gm); NaCl (0.1 g); and yeast extract (1.0 g) for 1L medium. A Whatman filter paper was placed on the bacterial growth. Then, the filter papers were saturated with a few drops of Salkowski reagent (1mL 0.5 M $FeCl_3$, 50 mL H_2SO_4). After two minutes, the appearance of pink colour was observed, an indicator of IAA production (Hemraj et al. 2013; AL-Joda and Jasim 2021).

Determination of nitrogen-fixation potential of isolated NFB strains

Screening of nitrogen-fixing activity of NFB

The nitrogen-fixing activity of NFB strains was assessed using solid Jensen's medium supplemented with 0.5% w/v of Bromothymol Blue (BTB) as a colour indicator. After an incubation period of 3–8 days, a colour change in the medium from greenish blue to dark blue was indicative of nitrogen-fixing activity. This colour alteration reflects the ability of the bacterial strains to fix nitrogen (Sulistiyan and Meliah 2017).

Ammonia production of NFB using Nessler's reagent

The nitrogen-fixation capacity of bacterial strains was assessed using Nessler's reagent. The strains were grown in 50 ml of 1 % peptone water under shaking at 100 rpm at 30°C for 3, 5, 7, and 9 days. After incubation, the culture broth was centrifuged at 4,000 rpm for 20 minutes, and the supernatant was carefully collected. A volume of 0.5 mL of Nessler's reagent (density 1.097 g/mL, 0.09 M HgI₂ and 0.5 M KI) was added to 1 mL of the supernatant. The development of colour, ranging from yellowish to brown, indicated the presence of ammonia in the sample, signifying nitrogen-fixing activity (Shomi et al. 2021).

Determination of ammonia, nitrite and nitrate production

The nitrogen fixation activity of NFB strains was determined following the method described by Ha and Chu (2020) with slight modification. Exactly 50 mL of Ashby broth media in sterilized flasks was prepared, and isolated NFB strains were inoculated into each flask separately. The broth media were then kept in a rotary shaker at 160 rpm for a 7-day of incubation. After the 7-day incubation period, the broth media's ammonia, nitrite, and nitrate levels were analyzed using standard spectrophotometry methods at 640 nm, 550 nm, and 420 nm, respectively.

Genotypic identification: Fungal strains and bacterial strains

The DNA extraction was performed using the Wizard Genomic DNA Purification Kit according to the manufacturer's protocol. Subsequently, the 16S rRNA gene was subjected to sequencing at Macrogen, Korea.

Phylogenetic Tree Construction

The phylogenetic tree was constructed using MEGA software based on 16S rRNA gene sequences. Sequence alignment was performed using the CLUSTALW algorithm, and the tree was generated using the Neighbour-Joining method

with 1,000 bootstrap replicates to assess branch reliability.

Determination of the effect of coir dust ratio and isolated NFB on plant growth

The effect of the coir dust ratio was determined under four growth medium compositions, as detailed below (Table 1). The growth medium for the pot experiment consisted of a blend of compost, coir dust, and soil.

Table 1 Prepared four types of growth medium (potting mixture)

Potting mixture	Composition (Soil: Coir dust: Compost)
Ratio 1	1 : 1 : 1
Ratio 2	1 : 0.75 : 1
Ratio 3	1 : 0.5 : 1
Ratio 4	1 : 0.25 : 1

Each pot was prepared using 200 grams of the homogenized growth medium. Then, each potting mixture was inoculated with NFB inoculants (each at a concentration of 10⁸ CFU/mL). Mung bean (*Vigna radiata*) plants were selected as the experimental plants for primary studies. 10 mL of each inoculant was added to pots containing sterilized media for mung bean seed growth. The bacterial inoculants were supplied only once at the beginning of the experiment. A control pot without inoculants treated with 10 mL of sterilized water, was included. The pot experiment was conducted in triplicate for both the NFB strain-treated pots and the control pots, which contained media without bacterial inoculation. The pots were rinsed twice daily with 50 mL of water per day. All treated and control pots were maintained under normal ambient environmental conditions, as the primary objective of the study was to assess field applicability. After the 30-day-old seed germination period, plant growth parameters, including shoot length, root length, wet weight and dry weight, were recorded to evaluate the impact of the selected strains on plant growth. The experiment followed a completely randomized block design with three replications per treatment.

Statistical analysis

Data analysis and graphical representations were conducted using Microsoft Excel. Data from different treatments were calculated and statistically analyzed with a one-way ANOVA using Python 3.11 (VS Code). The ANOVA test was performed to assess significant differences among treatment means. A significance level of $p < 0.05$ was used as the threshold to determine statistical significance.

Results and discussion

The NFB were successfully isolated and characterized from compost soil, and the effect of varying coir dust ratios on plant growth was examined. Coir dust was selected as a component of the growth medium due to its high water-holding capacity, good aeration properties, and ability to improve soil structure. Nitrogen fixation by bacteria is crucial for soil fertility and plant productivity, serving as an effective biofertilizer.

Table 2 Physicochemical properties of collected soil samples

Parameter	Compost Soil
Soil pH	3.13±0.02
Soil moisture (%)	56.70±1.2
Soil total organic carbon(%)	53.20±2.3
Soil total organic nitrogen (mg/L)	0.46±0.01

The physicochemical analysis of the compost soil, summarized in Table 2, indicated a low pH, high soil moisture, total organic carbon, and total organic nitrogen. These conditions provide a conducive environment for NFB strains. Acidic conditions often favor the proliferation of specific microbial communities, including NFB, which thrive in such environments. Elevated levels of total organic carbon and total organic nitrogen suggest substantial organic matter decomposition and nutrient cycling, characteristics of active microbial processes, including nitrogen fixation (Grzyb et al. 2021; Sun et al. 2021). Genotypic identification confirmed that among the isolated NFB strains (NFB-1 to NFB-7), five strains belonged to the genus *Bacillus*, while one strain was identified as belonging to the genus *Microbacterium* (Table 3). Previous studies

have demonstrated the nitrogen-fixing potential of *Bacillus* spp. and *Microbacterium* spp., supporting our findings (Jain et al. 2021; Zhang et al. 2022).

Table 3 Isolated NFB strains

Isolated strain	Strain name
NFB-1	<i>Microbacterium aquimaris</i>
NFB-2	<i>Bacillus xiamenensis</i> (MCCC 1A00008)
NFB-3	<i>Bacillus subtilis</i> (NCIB 3610)
NFB-4	<i>Bacillus cereus</i> (CCM 2010)
NFB-5	<i>Bacillus aerophilus</i> (XA5-12)
NFB-6	<i>Bacillus subtilis</i> (BHU7)
NFB-7	<i>Bacillus aerius</i> (24K)

The evolutionary relationships of isolated bacterial strains capable of nitrogen fixation among various *Bacillus* species and *Microbacterium aquimaris* are illustrated in Figure 1. The close genetic relationship among *Bacillus xiamenensis* (MCCC 1A00008) and *Bacillus aerophilus* (XA5-12) suggests they may share functional traits related to nitrogen fixation. Similarly, *Bacillus subtilis* (NCIB 3610) and *Bacillus subtilis* (BHU7) cluster together, indicating potential similarities in their nitrogen-fixing abilities. *Bacillus cereus* (CCM 2010) diverges earlier, suggesting some evolutionary differentiation in its nitrogen-fixing function (Yang et al. 2023). *Microbacterium aquimaris* is more distantly related as an outgroup, suggesting distinct mechanisms of nitrogen fixation compared to the *Bacillus* species (Ringø et al. 2008; Liu et al. 2016). Previous studies have shown that *Bacillus* spp. can effectively fix nitrogen, supporting their application as biofertilizers to enhance soil fertility (Nosheen et al. 2021). The evolutionary relationships observed emphasize the potential of *Bacillus* spp. and *Microbacterium* spp. strains in sustainable agricultural practices through nitrogen fixation.

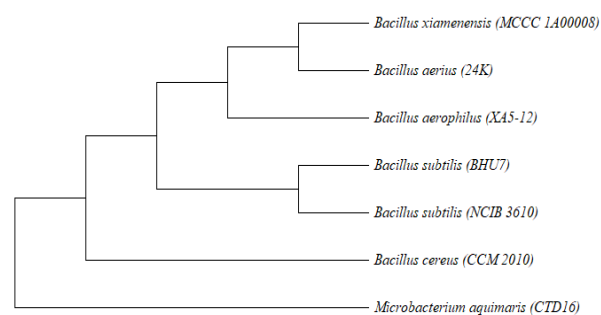


Figure 1 The evolutionary relationships among isolated NFB strains

Table 4 Biochemical results of isolated NSB strains

Bacterial strains	Biochemical test						
	Catalase test	Gram staining test	Motility test	Starch hydrolyses test	Spore Staining	Citrate Utilization Test	Spot Test Indole Production
<i>Microbacterium aquimaris</i>	+	+	-	-	-	+	-
<i>Bacillus xiamenensis</i> (MCCC 1A00008)	+	+	+	+	+	-	-
<i>Bacillus subtilis</i> (NCIB 3610)	+	+	+	+	+	-	-
<i>Bacillus cereus</i> (CCM 2010)	+	+	+	+	+	-	-
<i>Bacillus aerophilus</i> (XA5-12)	+	+	+	+	+	-	-
<i>Bacillus subtilis</i> (BHU7)	+	+	+	+	+	-	-
<i>Bacillus aerius</i> (24K)	+	+	+	+	+	-	-

(+ positive, - negative)

Furthermore, previous studies have shown that phylogenetic analysis of soil microbes underlines their multifunctional roles in sustainable agriculture by emphasizing that they promote plant growth through ammonia production, siderophore synthesis, and phosphorus solubilization (Bramhachar et al. 2027; Verma & Saharan 2020). The biochemical test results of isolated NFB strains are illustrated in Table 4. The positive catalase test suggests that these bacteria can decompose hydrogen peroxide, protecting themselves from oxidative stress. It benefits their survival and functionality in soil environments. The ability to hydrolyze starch implies that these bacteria can utilize starch as a carbon source, contributing to their growth and metabolic activity. It can enhance their nitrogen-fixing capabilities and thus promote plant growth. The formation of endospores indicates their ability to withstand harsh environmental conditions, ensuring their persistence in the soil and consistent nitrogen fixation. The positive citrate utilization test suggests that this strain can use citrate as a carbon source, enhancing its adaptability and survival in diverse soil conditions. The negative results for the other tests indicated that *Microbacterium aquimaris* has a more specialized metabolic profile compared to *Bacillus* spp., which might influence its specific role in nitrogen fixation and its interaction with plant roots (Hemraj et al. 2013; Talaiekhosani et al. 2015; AL-Joda and Jasim 2021; Shah et al. 2021).

Table 5 summarizes the nitrogen-fixing activity, ammonia production, and concentrations of nitrogenous compounds produced by isolated NFB strains. All isolated strains shown positive results

for nitrogen-fixing activity and ammonia production, indicating their potential as efficient biofertilizers to enhance nitrogen availability and promote plant growth. All strains produced ammonia, nitrite, and nitrate in Ashby broth medium after 7 days of incubation, confirming their nitrogen-fixing capabilities. The ammonia production indicates that these bacteria can convert atmospheric nitrogen into a form that plants can readily assimilate. The recorded ammonia (1.57 ± 0.05 mg/L) and nitrate (1.09 ± 0.04 mg/L) concentration in the Ashby broth medium was highest for the *Bacillus cereus* strain. *Bacillus subtilis* BHU7 produced more nitrate (0.99 ± 0.08 mg/L) compared to the other isolated strains. The positive results for nitrate and nitrite are attributed to the bacteria's inherent metabolic processes. NFB like *Bacillus* spp. and *Microbacterium aquimaris* can fix atmospheric nitrogen into ammonia and participate in nitrification pathways where ammonia is oxidized to nitrite and subsequently to nitrate. This sequential transformation can occur within the same bacterial strains due to specific enzymes facilitating these reactions. These isolated strains' production of ammonia, nitrite, and nitrate demonstrates their comprehensive role in nitrogen cycling within the soil (Oleńska et al. 2020; Kaur et al. 2021). Ammonia serves as a direct nitrogen source for plants. At the same time, nitrite and nitrate are critical for long-term nitrogen availability, as they are more stable and can be easily absorbed by plant roots. The ability of these bacteria to produce multiple forms of nitrogen enhances their effectiveness as biofertilizers, supporting sustainable plant growth and soil fertility (Paśmionka et al. 2021). The potential of

Bacillus cereus in ammonia and nitrate production may be attributed to its nitrogenase activity and efficient expression of enzymes involved in nitrogen fixation. Its reason for the conversion process of atmospheric nitrogen into plant-

accessible forms and supporting its role as a potent biofertilizer (Zheng et al. 2024). Previous studies have also demonstrated the potential of *Bacillus* spp. and *Microbacterium* spp. to fix nitrogen effectively (Satapute et al. 2012; Zheng et al. 2024)

Table 5 Nitrogen-fixing ability, ammonia production, and concentrations of nitrogenous compounds (nitrate, nitrite, and ammonia) produced by isolated NFB strains

Bacterial strains	Nitrogen fixing activity	Ammonia production	Nitrate (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)
<i>Microbacterium aquimaris</i>	+	+	0.96±0.04	0.033±0.001	0.26±0.02
<i>Bacillus xiamenensis</i> (MCCC 1A00008)	+	+	0.78±0.04	0.030±0.001	0.43±0.03
<i>Bacillus subtilis</i> (NCIB 3610)	+	+	0.96±0.04	0.062±0.002	0.62±0.04
<i>Bacillus cereus</i> (CCM 2010)	+	+	1.09±0.04	0.047±0.003	1.57±0.05
<i>Bacillus aerophilus</i> (XA5-12)	+	+	0.90±0.04	0.064±0.002	0.36±0.06
<i>Bacillus subtilis</i> (BHU7)	+	+	0.99±0.08	0.071±0.003	0.47±0.03
<i>Bacillus aerius</i> (24K)	+	+	0.90±0.04	0.057±0.002	0.51±0.05

(+ positive)

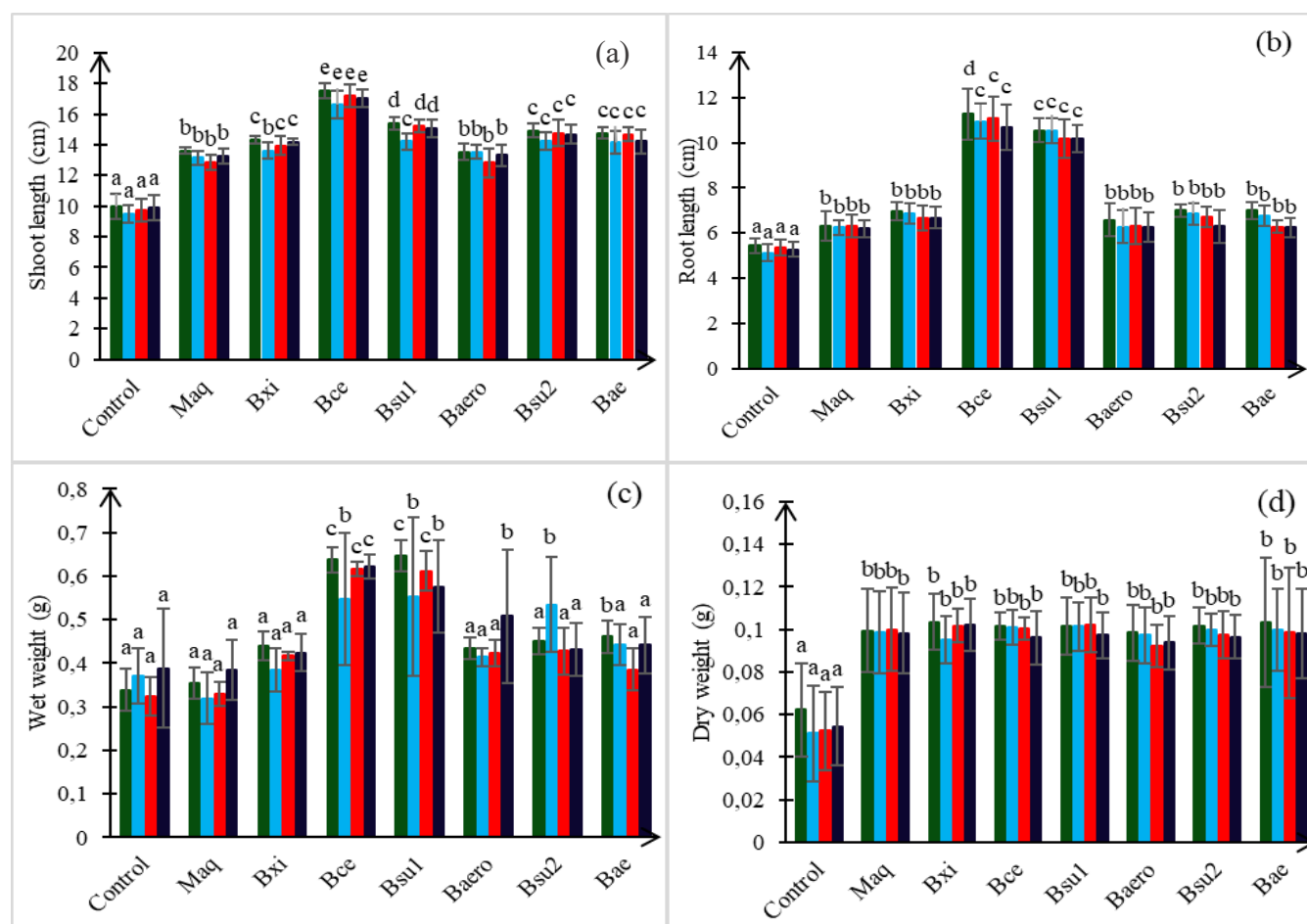


Figure 2: Effect of NFB strains on (a) shoot length, (b) root length, (c) wet weight and (d) dry weight of Mung bean (*Vigna radiata*) plants; Maq - *Microbacterium aquimaris*; Bxi - *Bacillus xiamenensis* (MCCC 1A00008); Bsu1 - *Bacillus subtilis* (NCIB 3610); Bce - *Bacillus cereus* (CCM 2010); Baero - *Bacillus aerophilus* (XA5-12); Bsu2 - *Bacillus subtilis* (BHU7); Bae - *Bacillus aerius* (24K); ■ – ratio 1; ■ – ratio 2; ■ – ratio 3; ■ – ratio 4.

The pot experiment revealed that the potting media consisting of a 1:1:1 ratio of compost, coir dust, and soil (Ratio 1) exhibited superior growth parameters, including shoot length, root length, wet weight, and dry weight (Figure 2).

A one-way ANOVA test confirmed that the potting media inoculated with *Bacillus cereus* (CCM 2010) (17.49 ± 0.50 cm), *Bacillus subtilis* NCIB 3610 (15.38 ± 0.41 cm), and *Bacillus subtilis* BHU7 (14.93 ± 0.42 cm) exhibited significantly greater shoot lengths compared to the media inoculated with other strains ($F(7, 64) = 181.27$, $p < 0.05$). Similarly, the potting media inoculated with *Bacillus cereus* (CCM 2010) (11.27 ± 1.12 cm) and *Bacillus subtilis* NCIB 3610 (10.54 ± 0.52 cm) showed significantly longer root lengths than those treated with other strains ($F(7, 64) = 106.88$, $p < 0.05$). Furthermore, the potting media inoculated with *Bacillus cereus* (CCM 2010) (0.6333 ± 0.02 g) showed higher wet weight than the growth media inoculated with other strains. However, there was no proper variation in the dry weight of plants grown in the potting media inoculated with different NFB strains. In a similar study, Chandra et al. (2018) identified *B. subtilis* (BSSC11) and *B. megaterium* (BMSE7) strains, which improved sugarcane growth (high shoot and root weight) by enhancing nutrient content. These findings emphasize the potential of NFB strains to enhance plant growth and improve nutrient content and stress resilience in various crops, reinforcing their application as biofertilizers in sustainable agricultural practices. Da Costa Neto et al. (2024) found that *Bacillus pumilus* significantly promoted plant growth and enhanced biological nitrogen fixation in mung bean. Its application increased shoot biomass, nitrogen accumulation, and the concentration of nitrogenous compounds, indicating its strong potential as an effective nitrogen-fixing biofertilizer. Moreover, Pataczek et al. (2024) reported that single-strain inoculation with *Bacillus subtilis* significantly enhanced mungbean root biomass, total dry matter, and total plant nitrogen accumulation, while multi-strain inoculation showed no such effect.

These findings emphasize the potential of specific NFB strains like *B. subtilis* to improve nutrient uptake and plant productivity, supporting their application as targeted biofertilizers in sustainable

agriculture. Similarly, our study focused on evaluating the effects of single-strain inoculation on plant growth and confirmed the positive impact of individual NFB strains. However, the potential synergistic effects of multi-strain consortia require further investigation to optimize biofertilizer formulations and maximize plant growth benefits.

The growth parameters of the potting mixture demonstrated a significant difference ($p < 0.05$) compared to the control potting media across all examined ratios. Additionally, the potting mix inoculated with *Microbacterium aquimaris* improved measured growth parameters for all ratios compared to the control growth media. These findings suggest that *Bacillus* spp. strains and *Microbacterium aquimaris* play crucial roles in improving plant growth through improved nitrogen fixation and nutrient availability. This study was conducted under ambient environmental conditions, providing a practical evaluation of the isolated NFB strains on mung bean growth. However, the 30-day duration reflects an early growth phase, which may limit understanding of long-term effects on plant development and yield. Long-term evaluation through field studies in diverse environmental conditions is crucial to validate the efficacy and consistency of these biofertilizers in sustainable agriculture.

However, ecological risks associated with the application of *Bacillus cereus* in agriculture should be focused on, as some strains of this species are known to be pathogenic to humans and animals. In this study, all isolated *Bacillus* strains were confirmed as non-pathogenic. Careful strain selection and thorough safety assessments are necessary to mitigate any potential health hazards while utilizing *Bacillus cereus* as a biofertilizer. The high organic content and acidic pH of the compost site soils likely provided a conducive environment for these strains, further supporting their effectiveness as biofertilizers in sustainable agriculture. A 1:1:1 ratio of compost, coir dust, and soil is recommended for general crop applications due to its consistent support of plant growth. The combination of compost, coir dust, and soil in a balanced ratio ensures adequate aeration, water retention, and nutrient supply, essential for optimal microbial activity and plant growth. Future research should focus on large-scale field trials to

validate the effectiveness of these microbial biofertilizers under diverse environmental conditions.

Conclusions

The present study isolated and characterized NFB strains from compost soil and evaluated the impact of different coir dust ratios on plant growth. The isolated strains, including *Bacillus cereus* CCM 2010, *Bacillus subtilis* NCIB 3610, *Bacillus subtilis* BHU7, and *Microbacterium aquimaris*, demonstrated significant potential in increasing plant growth parameters such as shoot length, root length, wet weight, and dry weight. Notably, the potting media consisting of a 1:1:1 ratio of compost, coir dust, and soil inoculated with these strains showed superior growth performance compared to other ratios and the control setup. The physicochemical analysis of the compost soil revealed an acidic pH and high levels of total organic carbon and nitrogen, which provided a favorable environment for the proliferation of NFB. The isolated *Bacillus* spp. and *Microbacterium aquimaris* strains exhibited key biochemical traits, including catalase activity, motility, spore formation, and starch hydrolysis, indicating their robust metabolic capabilities and adaptability to soil environments. The isolated strains' production of ammonia, nitrite, and nitrate highlights their comprehensive role in nitrogen cycling, contributing to soil fertility and plant nutrition. The pot experiment further confirmed the beneficial effects of these NFB strains on mung bean growth, with *Bacillus cereus* CCM 2010 and *Microbacterium aquimaris* showing the most pronounced improvements in growth parameters. These findings emphasize the potential of utilising *Bacillus* spp. and *Microbacterium aquimaris* as effective biofertilizers in sustainable agriculture. Integrating compost, coir dust, and soil in a balanced ratio provides an optimal growth medium that supports microbial activity and enhances nutrient availability. Future research should focus on long-term trials under field conditions and different soil conditions to confirm the reliability and effectiveness of the observed plant growth-promoting effects.

Acknowledgments

The authors would like to acknowledge the Research Council of the University of Sri Jayewardenepura, Sri Lanka, for supporting the study under the Research Grant ASP/01/RE/SCI/2022/36, and the Centre for Water Quality and Algae Research for providing the laboratory facilities for this study.

References

- AL-Joda BMS, Jasim AH (2021). Biochemical testing revision for identifying several kinds of bacteria. *Journal of University of Babylon for Pure and Applied Sciences*, 29(2): 168-176.
- AOAC (2000) Official methods of analysis of AOAC International 17th ed. Gaithersburg, MD, USA
- Atzori G, Pane C, Zaccardelli M, Cacini S, Massa D (2021) The role of peat-free organic substrates in the sustainable management of soilless cultivations. *Agronomy* 11(6): 1236.
- Bamdad H, Papari S, Lazarovits G, Berruti F (2022) Soil amendments for sustainable agriculture: Microbial organic fertilizers. *Soil Use and Management* 38(1): 94-120
- Bramhachari PV, Nagaraju GP, Kariali E (2017) Metagenomic approaches in understanding the mechanism and function of PGPRs: perspectives for sustainable agriculture. In *Agriculturally Important Microbes for Sustainable Agriculture: Volume I: Plant-soil-microbe nexus*, pp.163-182.
- Chandra P, Tripathi P, Chandra A (2018) Isolation and molecular characterization of plant growth-promoting *Bacillus* spp. and their impact on sugarcane (*Saccharum* spp. hybrids) growth and tolerance towards drought stress. *Acta Physiologiae Plantarum* 40(11): 199.
- Chromkaew Y, Kaeomuangmoon T, Mawan N, Mukjang N, Khongdee N (2023) Is coconut coir dust an efficient biofertilizer carrier for promoting coffee seedling growth and nutrient uptake? *PeerJ* 11: e15530.
- da Costa Neto VP, de Melo ARP, Alencar CES, de Lima VBC, Zilli JE, Rodrigues AC, Bonifacio A (2024) Bacterial consortia among *Bradyrhizobium* species, *Azospirillum baldaniorum* and *Bacillus pumilus* promote plant growth and efficient symbiotic nitrogen fixation in mung bean. *Symbiosis* 93(3): 255–267.
- Grzyb A, Wolna-Maruwka A, Niewiadomska A (2021) The significance of microbial transformation of nitrogen compounds in the light of integrated crop management. *Agronomy* 11(7): 1415.
- Hamza TA, Hussein Z, Mitku R, Ayalew P, Belayneh T (2017) Isolation and characterization of nitrogen fixing bacteria from rhizosphere soil collected from Shell Mele agricultural center, southern Ethiopia. *J Agric Sci Food Technol* 3(7): 117-124.
- Hemraj V, Diksha S, Avneet G (2013) A review on commonly used biochemical test for bacteria. *Innovare Journal of Life Science* 1(1): 1-7.

- Jain S, Varma A, Choudhary DK (2021) Perspectives on nitrogen-fixing *Bacillus* species. In Soil Nitrogen Ecology pp.359-369.
- Jayathilake KMPI, Manage PM, Idroos FS (2025) Isolation and characterization of phosphate-solubilizing bacteria from compost and open dump sites: Implications for plant growth. Nature Environment and Pollution Technology 24(3)
- Jayathilake P, Manage P, Idroos F (2026) Synergistic effect of *Bacillus* spp. and *Aspergillus* spp. as biofertilizers spray on nutrient uptake and growth enhancement in lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*): Impact of biofertilizer on soil fertility and sustainable agriculture. Journal of Microbiology, Biotechnology and Food Sciences 15(4): e12404.
- Kaur T, Devi R, Kour D, Yadav A, Yadav AN, Dikilitas M, Abdel-Azeem AM, Ahluwalia AS, Saxena AK (2021) Plant growth promoting soil microbiomes and their potential implications for agricultural and environmental sustainability. Biologia 76(9): 2687-2709.
- Kumar PS, Rangasamy G, Gayathri KV, Parthasarathy V (2023) Rhizobium mayense sp. Nov., an efficient plant growth-promoting nitrogen-fixing bacteria isolated from rhizosphere soil. Environmental Research 220: 115200.
- Liu Y, Lai Q, Shao Z (2016) Identification of “*Bacillus cellulosensis*” strain NIO-1130 T as a member of *Bacillus altitudinis* and emendation of the latter. Archives of Microbiology 198: 835-838.
- Monib AW, Niazi P, Barai SM, Sawicka B, Baseer AQ, Nikpay A, Fahmawi SMS, Singh D, Alikhail M, Thea B (2024) Nitrogen cycling dynamics: Investigating volatilization and its interplay with N₂ fixation. Journal for Research in Applied Sciences and Biotechnology (1): 17-31.
- Mussa SAB, Elferjani HS, Haroun FA, Abdelnabi FF (2009) Determination of available nitrate, phosphate and sulfate in soil samples. International Journal of PharmTech Research 1(3): 598-604.
- Nosheen S, Ajmal I, Song Y (2021) Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability 13(4): 1868.
- Oleńska E, Małek W, Wójcik M, Swiecicka I, Thijs S, Vangronsveld J (2020) Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. Science of the Total Environment 743: 140682.
- Paśmionka IB, Bulski K, Boligłowa E (2021) The participation of microbiota in the transformation of nitrogen compounds in the soil—A review. Agronomy 11(5): 977.
- Pataczek L, Armas JCB, Petsch T, Hilger T, Ahmad M, Schafleitner R, Zahir ZA, Cadisch G (2024) Single-strain inoculation of *Bacillus subtilis* and *Rhizobium phaseoli* affects nitrogen acquisition of an improved mungbean cultivar. Journal of Soil Science and Plant Nutrition 24(4): 6746–6759.
- Ringø E, Sperstad S, Kraugerud OF, Krogdahl Å (2008) Use of 16S rRNA gene sequencing analysis to characterize culturable intestinal bacteria in Atlantic salmon (*Salmo salar*) fed diets with cellulose or non-starch polysaccharides from soy. Aquaculture Research 39(10): 1087-1100.
- Sammauria R, Kumawat S, Kumawat P, Singh J, Jatwa TK (2020) Microbial inoculants: potential tool for sustainability of agricultural production systems. Archives of Microbiology 202(4): 677-693.
- Satapute PP, Olekar HS, Shetti AA, Kulkarni AG, Hiremath GB, Patagundi BI, Shivsharan CT, Kaliwal BB (2012) Isolation and characterization of nitrogen-fixing *Bacillus subtilis* strain AS-4 from agricultural soil. International Journal of Recent Scientific Research 3(9): 762–765.
- Shah D, Khan MS, Aziz S, Ali H, Pecoraro L (2021) Molecular and biochemical characterization, antimicrobial activity, stress tolerance, and plant growth-promoting effect of endophytic bacteria isolated from wheat varieties. Microorganisms 10(1): 21.
- Sharma P, Bano A, Singh SP, Tong YW (2023) Microbial inoculants: Recent progress in formulations and methods of application. In Microbial Inoculants: 1-28. <https://doi.org/10.1016/B978-0-323-99043-1.00017-7>
- Shomi FY, Uddin MB, Zerin T (2021) Isolation and characterization of nitrogen-fixing bacteria from soil sample in Dhaka, Bangladesh. Stamford Journal of Microbiology 11(1): 11–13
- Sivaram AK, Abinandan S, Chen C, Venkateswarlu K, Megharaj M (2023) Microbial inoculant carriers: Soil health improvement and moisture retention in sustainable agriculture. Advances in Agronomy 180: 35-91.
- Sulistiyan TR, Meliah S (2017) Isolation and characterization of nitrogen-fixing endophytic bacteria associated with sweet sorghum (*Sorghum bicolor*). In Proceedings of The SATREPS Conference 1(1): 110–117.
- Sun WENLI, Shahrajabian MH, Cheng QI (2021) Nitrogen fixation and diazotrophs—a review. Rom Biotechnol Lett 26(4): 2834-2845.
- Talaiekhazani A, Alaee S, Ponraj M (2015) Guidelines for quick application of biochemical tests to identify unknown bacteria. AOB 2(2): 65-82.
- Vats S, Srivastava P, Saxena S, Mudgil B, Kumar N (2021) Beneficial effects of nitrogen-fixing bacteria for agriculture of the future. In Soil Nitrogen Ecology, Cham: Springer International Publishing: 305-325.
- Verma S, Saharan BS (2020) 16S rRNA phylogenetic analysis of heavy metal tolerant plant growth promoting rhizobacteria. Nature Environment and Pollution Technology 19(4): 1763-1766.
- Walkley A, Black IA (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid in soil analysis. Soil Sci 79: 459-465.
- Yang X, Xie Y, Qiao Y, Chen L, Wang T, Wu L, Li J, Gao Y (2023) Analysis of the biological activity and whole genome sequencing of *Bacillus cereus* CDHWZ7 isolated from the rhizosphere of *Lycium ruthenicum* on the Tibetan plateau. Agriculture 13(5): 1041.
- Zhang X, Tong J, Dong M, Akhtar K, He B (2022) Isolation, identification and characterization of nitrogen fixing

- endophytic bacteria and their effects on cassava production. PeerJ 10: e12677.
- Zheng J, Liao Y, Li Y, Li D, Sun Y, Xiao Z (2024) Description of *Microbacterium dauci* sp. nov., a plant growth hormone indoleacetic acid-producing and nitrogen-fixing bacterium isolated from carrot rhizosphere soil. Archives of Microbiology 206(3): 102.