



Enhancing Oil Palm (*Elaeis guineensis* Jacq.) Growth Using Microbial Consortia: Roles of Phosphate Solubilization and Nitrogen Fixation

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Abstract: In Thailand, oil palm is a vital economic crop, primarily cultivated in the southern region. Farmers are expanding plantations by establishing new sites and replanting previous crop areas. Proper maintenance from the seedling stage is essential for growth. Agriculturists place rock phosphate at the planting hole base to speed seedling recovery after transplantation. Converting rock phosphate into water-soluble phosphate, essential for root development and pre-planting, is a labor-intensive process dependent on microbial activity. The objective of this investigation is to identify microbes capable of converting insoluble phosphate into a soluble form in water. Furthermore, oil palm seedlings require a significant amount of nitrogen. Therefore, nitrogen-fixing microorganisms are also screened. Several phosphate-soluble microorganisms have been identified, especially the NN311 strain, which is the most effective at releasing water-soluble phosphate, both qualitatively and quantitatively. For nitrogen fixation, strain NT7 performed best. NN311 was classified as *Pseudomonas* sp., whereas NT7 was classified as *Bacillus* sp., according to nucleic acid sequence analysis in comparison to the NCBI database. In the absence of antagonistic interactions between NN311 and NT7, they were assessed simultaneously in nursery research. The results indicated that the application of these microorganisms, individually or in combination, markedly enhanced seedling growth compared to the control group. Nonetheless, combining them yields optimal outcomes. The combination of NN311 and NT7 with rock phosphate in the planting hole enhances seedling growth post-transplantation. After 18 months, seedlings treated with both strains of bacteria exhibit much superior growth compared to those that do not receive microorganisms.

Keywords: Oil palm seedling; plant growth promotion; phosphorus releasing; nitrogen fixation

1. Introduction

The oil palm (*Elaeis guineensis* Jacq.) is a crop of significant economic value that has received government backing to incentivize farmers to increase its cultivation in response to the energy crisis in Thailand and globally.

Moreover, oil palm products can be used in many consumer and industrial products, making it a crucial crop for the nation in line with national strategies outlined in the Oil Palm Industry Development Plan. Furthermore, Thailand's tropical environment is well-suited to oil palm cultivation, giving it a competitive edge over alternative oil-producing crops. Southern Thailand hosts the majority of oil palm farms, covering 0.86 million ha out of a total of 1 million ha [1]. Despite government subsidies, domestic oil palm production falls short of meeting demand, and we anticipate rising demand for palm oil products in the future. Agricultural producers face difficulties, including price fluctuations, rising production costs, and climate change, which lead to income instability. To augment oil palm output, two primary strategies can be employed: expanding cultivation areas and enhancing yield per unit area. Cultivating high-yielding cultivars with elevated oil content can enhance yield per unit area. Fertilization is crucial, and chemical fertilizers are predominantly preferred. Prolonged application of chemical fertilizers results in environmental degradation, diminishes soil fertility, reduces beneficial microbes and soil-dwelling creatures, and disrupts ecological equilibrium. It also escalates production expenses. To alleviate these challenges and decrease expenses, the utilization of plant growth-promoting microorganisms offers a viable solution. Farmers can economically produce microbial inoculants, offering a sustainable approach to increasing oil palm yield while minimizing environmental impact.

Plant Growth-Promoting Rhizobacteria (PGPRs) are increasingly employed in contemporary agriculture owing to their capacity to stimulate plant growth through direct and indirect mechanisms. These advantageous root-colonizing bacteria enhance plant growth by fixing atmospheric nitrogen, solubilizing vital nutrients such as phosphorus and potassium, and synthesizing siderophores that augment iron availability. Furthermore, PGPRs excrete phytohormones that promote cell division and elongation in roots, shoots, and leaves, and accelerate seed germination, flowering, and fruiting [2]. They indirectly inhibit plant pathogens by competitive exclusion and stimulate systemic resistance in plants. Prevalent genera of PGPRs encompass *Pseudomonas*, *Bacillus*, *Enterobacter*, and *Erwinia* [3]. The PGPRs isolated from the oil palm rhizosphere have exhibited considerable growth-promoting properties, including nitrogen fixation, enhanced nutrient uptake, and improved root and shoot development [4, 5]. The PGPRs have demonstrated encouraging results in enhancing yields of several crops, such as peanuts, canola, safflower, and rapeseed, underscoring their potential for sustainable agricultural use [6, 7, 8, 9].

This research investigates the impact of microbial inoculation on the physical and biological alterations during the initial growth phase of oil palm seedlings. Despite increasing interest in beneficial microorganisms in agriculture, a substantial information gap persists regarding the application of microbial consortia, particularly in oil palm cultivation during the nursery stage, where such methods are either restricted or not yet implemented. Therefore, this study addresses these unresolved questions by: (1) evaluating the impact of selected PGPR strains on the physical and biological growth parameters of oil palm seedlings during the nursery stage; (2) identifying microbial strains or consortia that most effectively enhance germination, root development, shoot elongation, and seedling vigor in oil palm; and (3) giving insight into the potential of PGPRs as a sustainable alternative to chemical fertilizers in oil palm nurseries. The results will aid the development of more effective and sustainable cultivation methods for oil palm seedlings, potentially reducing dependence on chemical inputs while enhancing seedling quality and uniformity. This study is especially important given the rising global demand for palm oil and the need for more sustainable agricultural practices.

2. Materials and Methods

2.1 Oil Palm Seedling Preparation

The experiment used oil palm seeds of the Tenera hybrid variety, obtained from the Suratthani Oil Palm Research Center, Department of Agriculture, Thailand. The seeds were surface sterilized before germination by being washed with 70% ethanol, submerged in 5% sodium hypochlorite for 10 min, and then rinsed with sterile distilled water. The sterilized seeds were kept at 30°C for 60 days to facilitate germination. After this period, germinating seedlings were sprayed with sterile distilled water and kept at 30°C for an

additional 7 days. Seeds with root lengths of 2.0–2.5 cm and shoot lengths of 1.0–1.5 cm [10] were selected and transplanted into 6 × 12-inch polyethylene bags containing 0.5 L of sterile peat moss.

2.2 Screening of PGPRs

Soil samples were collected from the rhizosphere of oil palms across eight different zones in Hat Yai District, Songkhla Province, Thailand. From each sample, 20 g of soil was placed in a 50 mL Erlenmeyer flask and vigorously shaken with 30 mL of sterile saline solution (0.85% NaCl) for 2 min. After shaking, the mixture was allowed to settle for one hour. The supernatant was then carefully decanted, while the sediment was discarded [11]. The resulting eluent was serially diluted 10-fold before being spread onto selective media for phosphate-solubilizing and nitrogen-fixing bacteria.

2.2.1 Phosphate Solubilization Capacity

Pikovskaya's agar plates (15 cm in diameter) were covered with a 100 µL aliquot of the diluted sample. Each liter of Pikovskaya's agar medium comprises 0.5 g (NH₄)₂SO₄, 0.2 g NaCl, 0.1 g MgSO₄·7H₂O, 0.002 g MnSO₄, 0.002 g FeSO₄·7H₂O, 0.2 g KCl, 0.5 g yeast extract, 10 g glucose, 5 g Ca₃(PO₄)₂, and 15 g agar with a pH adjusted to 7.0 ± 0.02. Bacterial colonies with clear zones showing phosphate dissolution can be transferred using aseptic loops into a 100 mL Erlenmeyer with 50 mL of NB media. The cultures were grown at 30°C with continuous agitation at 200 rpm for 24 h. Centrifugation was then used for 20 min at 8,000 rpm to obtain the bacterial cells. The pellet was rinsed twice with sterile distilled water and resuspended in sterile saline (0.85% NaCl) to achieve a turbidity corresponding to the 0.5 McFarland standard. One milliliter of the bacterial suspension was then added to 100 mL of Pikovskaya's broth, and the mixture was agitated (at 200 rpm) for 7 days at 30°C. After incubation, the culture was centrifuged (8,000 rpm, 20 min), and the supernatant was collected for quantification of soluble phosphate using KH₂PO₄ as the standard [12].

2.2.2 Nitrogen Fixation Capacity

A 100 µL diluted sample was applied to nitrogen-free agar medium. The inoculation plates were then incubated for 7 days at 37°C. Bacterial colonies that developed on the nitrogen-free medium were aseptically inoculated into 50 mL of nutrient broth using a sterile loop. All broths were subsequently incubated at 37°C with continuous agitation at 200 rpm for 24 hours. The bacterial cells were collected by centrifugation at 8,000 rpm for 20 min, rinsed twice with sterile distilled water, and then resuspended in sterile saline solution to achieve a turbidity matching the 0.5 McFarland standard. One mL of the bacterial suspension was introduced into 100 mL of nitrogen-free bromothymol blue medium [13] and cultured at 30°C with shaking at 200 rpm for 7 days. After incubation, the culture was centrifuged at 8,000 rpm for 20 min, and the supernatant was collected for total nitrogen analysis by the Kjeldahl method [14].

2.3 PGPRs identification

This study focused on identifying microbial strains that possess superior phosphate-solubilizing and nitrogen-fixing abilities. DNA was extracted from the microorganisms [15], and the 16S rRNA gene fragment was amplified by PCR using universal eubacterial primers (fD1 and rD1). The amplification approach consisted of an initial denaturation at 94°C for 1 min, followed by annealing at 55°C for 1 min, and extension at 72°C for 1 min, repeated for 30 cycles, terminating with a final extension at 72°C for 1 min [16]. The amplified 16S rRNA gene region was subsequently ligated into the pGEM-T easy vector for sequencing applications. The resulting nucleotide sequences were aligned with data in the NCBI database [17] to identify the microbial type precisely.

2.4 Microbial Compatibility Assessment

The compatibility of phosphate-solubilizing and nitrogen-fixing strains was evaluated using a modified cross-streak assay [18]. Nutrient agar plates (15 cm in diameter) were inoculated with 100 µL of strain 1 (adjusted to 10³ CFU mL⁻¹) and evenly distributed to get isolated colonies. A sterile filter paper strip (0.5 × 12 cm, Whatman No. 1) was aseptically placed on the inoculated medium, followed by the addition of 500 µL of strain 2 suspension (10³ CFU mL⁻¹) along the strip. Plates were incubated aerobically for 18 h at 37 ± 0.5°C. The inhibition zones at the interface between Strains 1 and 2 were examined to assess antagonism. Three biological replicates were performed for each strain combination.

2.5 Effect of PGPRs on oil palm seedlings in the nursery

The selected rhizobacteria that fix nitrogen and solubilize phosphate were cultivated in 100 mL of nutrient broth at 30°C while being continuously shaken at 200 rpm for 24 h. The bacterial cells were obtained by centrifugation (8,000 rpm, 20 min), rinsed twice with sterile distilled water, and then resuspended in PBS buffer (10 mM NaH₂PO₄ in 0.85% NaCl, pH 6.5) to reach a McFarland-standard concentration of 10⁹ CFU mL⁻¹. Oil palm seedlings were divided into four treatment groups: (1) bacteria that fix nitrogen, (2) bacteria that solubilize phosphate, (3) bacteria that fix nitrogen and solubilize phosphate, and (4) control (sterile distilled water). Two experimental runs, three replicates per treatment, and ten seedlings per replication were used in the investigation, which was conducted in the greenhouse at the Faculty of Natural Resources, Prince of Songkla University in Thailand, using a randomized complete block design (RCBD). Physical growth factors and levels of carotenoids and chlorophyll were monitored over the 5 months during which treatments were given monthly. At 5 months of age, seedlings underwent destructive analysis to measure: (1) biomass parameters: fresh weight (total, stem, root), dry weight (total, stem, root), and (2) morphological parameters: leaf length, total leaf number, root area, leaf area, and root: shoot ratio. The second leaf from the shoot apex was collected, flash-frozen in liquid nitrogen, and ground to a fine powder for pigment analysis. Exactly 0.2 g of leaf powder was extracted with 20 mL of 90% acetone in darkness [19]. Chlorophyll and carotenoid contents were determined spectrophotometrically at 663 nm, 647 nm, and 480 nm and calculated using the following equations:

$$\text{Chlorophyll a } (\mu\text{g cm}^{-2}) = [(12.64 \times A_{664} - 2.99 \times A_{647}) \times V] / (LA \times 100)$$

$$\text{Chlorophyll b } (\mu\text{g cm}^{-2}) = [(23.26 \times A_{647} - 5.06 \times A_{664}) \times V] / (LA \times 100)$$

$$\text{Total chlorophyll } (\mu\text{g cm}^{-2}) = [(20.27 \times A_{647} + 7.04 \times A_{664}) \times V] / (LA \times 100)$$

$$\text{Carotenoids } (\mu\text{g cm}^{-2}) = [(1000 \times A_{480} - 1.12 \times \text{Chl a} - 34.07 \times \text{Chl b}) / 245 \times V] / (LA \times 100)$$

Where:

A = Absorbance at specified wavelength

V = Solvent volume (mL)

LA = Leaf area (cm²)

Chl a = Chlorophyll a

Chl b = Chlorophyll b

2.6 Effect of PGPR on oil palm in the field

The field experiment utilized 14-month-old Deli × Ghana oil palm seedlings transplanted into clay soil (pH < 4.5, Total N < 100 ppm, Available P < 5 ppm, and organic matter 1%) in a 4.8 ha plantation in Ko Nang Kham Subdistrict, Phatthalung Province, Thailand (7.285094°N, 100.410708°E). The experiment used two co-selected microbial species known for their enhanced phosphorus solubility and nitrogen-fixation capabilities. Before planting the oil palm seedlings, prepare the base of each hole by mixing 500 mL of each microbial suspension (10⁹ CFU mL⁻¹) with 500 g of phosphate rock (0-3-0, P₂O₅ available = 3%) and 5 L of dehydrated cow manure. An RCBD experiment was conducted with four replications, each containing five seedlings. Control plots were included and received the same amendments, but did not receive microbial inoculation. Evaluate the growth performance by measuring plant height 18 months after cultivation to assess the effectiveness of microbial application on seedling formation and development in real growing conditions.

2.7 Statistical analysis

All experimental data were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, means were compared using Tukey's honest significant difference (HSD) post-hoc test. Results are presented as mean values ± standard error of the mean (SE) with three biological replicates (n = 3). A probability level of $p \leq 0.05$ was considered statistically significant for all analyses.

3. Results and Discussion

3.1 Microbial Selection

3.1.1 Screening for Phosphate-Solubilizing Microorganisms

The ability to transform insoluble phosphate into a water-soluble form is a key criterion for selecting microorganisms that promote plant growth. An examination of dishes using Pikovskaya's agar, which contains solid nutrients, reveals a milky-white color due to insoluble phosphate in the medium. Microorganisms were collected from various soil sources associated with plant roots. They were cultivated on Pikovskaya's agar at $30 \pm 5^\circ\text{C}$ for 7 days. Their presence was indicated by distinct, clear zones around the colonies, as shown in Figure 1; however, most of these zones were small, suggesting limited phosphate solubilization capacity. A few isolated microbial colonies exhibited substantial clear zones, suggesting their exceptional ability to convert phosphate into a water-soluble form. The microorganisms initially selected displayed phosphate solubilization efficiency values outlined in Table 1. Their ability to quantitatively convert phosphate into a water-soluble form was evaluated against a standard graph of KH_2PO_4 , as shown in Table 2. Colonies that strongly converted phosphate to its soluble form also showed higher quantitative values.

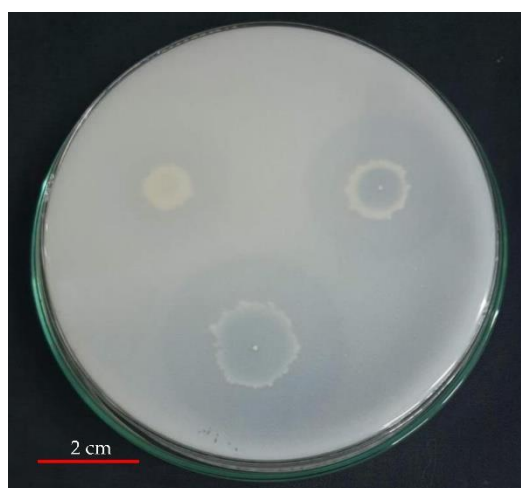


Figure 1. Microbial colonies exhibiting phosphate-solubilizing activity on Pikovskaya's agar medium, demonstrated by clear halo zones surrounding colonies.

Table 1. Qualitative assessment of phosphate solubilization efficiency by microbial isolates, as indicated by clear zone formation on Pikovskaya's agar medium

Microbial isolate	Phosphate Solubilization Efficiency (PSE)
D122	$2.53 \pm 0.15\text{b}$
BS	$2.23 \pm 0.12\text{a}$
D321	$2.00 \pm 0.00\text{a}$
NN321	$2.28 \pm 0.11\text{ab}$
D111	$2.32 \pm 0.19\text{ab}$
NN322	$2.42 \pm 0.04\text{b}$
NN311	$3.37 \pm 0.19\text{c}$

The table presents calculated PSE ratios from the measured clear zone diameters for each microbial strain, demonstrating their relative phosphate-solubilizing capabilities. Data represent mean $\pm$ SE. Means followed by the same lowercase letter do not differ significantly (Tukey's HSD test, $p \leq 0.05$).

Table 2. Quantitative analysis of phosphate solubilization capacity by selected microbial isolates

Microbial isolate	Total soluble phosphate ($\mu\text{g mL}^{-1}$)
D122	$0.60 \pm 0.07\text{b}$
BS	$0.31 \pm 0.00\text{a}$
D321	$0.54 \pm 0.02\text{b}$
NN321	$0.84 \pm 0.03\text{c}$
D111	$0.33 \pm 0.00\text{a}$
NN322	$0.30 \pm 0.00\text{a}$
NN311	$1.77 \pm 0.05\text{d}$

The table presents solubilized phosphate concentrations measured spectrophotometrically against a KH_2PO_4 standard curve. Data represent mean $\pm$ SE. Means followed by the same lowercase letter do not differ significantly (Tukey's HSD test, $p \leq 0.05$).

3.1.2 Screening for Nitrogen-Fixing Microorganisms

The nitrogen-fixing capacity of microbial isolates was evaluated using the Kjeldahl method. Among the tested isolates, 66.67% demonstrated nitrogen-fixing activity, with strain NT7 exhibiting the highest nitrogen fixation rate, fixing 1.1204 mg L^{-1} of nitrogen. Other notable isolates included: PSB1 (0.8870 mg L^{-1}), NT4 (0.8403 mg L^{-1}), PSB2 (0.7469 mg L^{-1}), BS (0.4668 mg L^{-1}), and NT3 (0.4202 mg L^{-1}). These quantitative results, summarized in Table 3, highlight NT7 as the most efficient nitrogen-fixing strain, suggesting strong potential to enhance nitrogen availability in agricultural systems.

Table 3. Nitrogen fixation capacity of isolated microbial strains quantified using the Kjeldahl method

Microbial isolate	Total N (mg L^{-1})
control	ND
NT3	$0.4202 \pm 0.0154\text{a}$
NT4	$0.8403 \pm 0.0755\text{b}$
NT7	$1.1204 \pm 0.0461\text{c}$
NT8	ND
BS	$0.4668 \pm 0.0034\text{a}$
PSB1	$0.8870 \pm 0.1084\text{b}$
PSB2	$0.7469 \pm 0.0649\text{b}$
NN311	ND
NN411	ND

Data represent mean $\pm$ SE. Means followed by the same lowercase letter do not differ significantly (Tukey's HSD test, $p \leq 0.05$). ND = Not Detected

3.2 Microbial Strain Identification

Upon selecting the most proficient phosphate-solubilizing and nitrogen-fixing isolates, microbial strain identification was conducted to confirm their genus. DNA extraction and PCR amplification of the 16S rRNA gene ($\sim 1,500 \text{ bp}$) employing universal primers (fD1/rD1) produced distinct fragments, which were then sequenced and analyzed using BLAST against the NCBI database. The phosphate-solubilizing isolate NN311 demonstrated significant sequence similarity to *Pseudomonas* sp., a genus extensively recognized for its capacity to solubilize inorganic phosphorus via organic acid secretion [20-22]. Simultaneously, the nitrogen-fixing isolate NT7 exhibited a strong association with *Bacillus* sp., a genus known for its capacity to function as a diazotroph in soil ecosystems. The molecular identifications validate the taxonomic classification of the strains and correspond with their presumed functions in nutrient cycling, as *Pseudomonas* and *Bacillus* species are often associated with improving soil fertility through phosphorus mobilization and atmospheric nitrogen fixation, respectively [23, 24, 25, 26]. The amalgamation of molecular and functional data underlines the promise of these isolates as bioinoculants for sustainable agriculture, especially in nutrient-deficient soils.

3.3 Microbial compatibility test

The cross-streak compatibility assay revealed the absence of antagonistic interactions between the nitrogen-fixing *Bacillus* sp. (NT7) and the phosphate-solubilizing *Pseudomonas* sp. (NN311), as shown by the absence of inhibition zones (Figure 2). The compatibility of these organisms is crucial for their potential combined application in agricultural systems, as microbial antagonism may diminish the efficacy of bioinoculants in agriculture. The observed coexistence indicates that these strains may engage in synergistic interactions rather than competitive exclusion, a characteristic that is advantageous for the formation of microbial consortia that provide various plant growth-promoting functions [27].

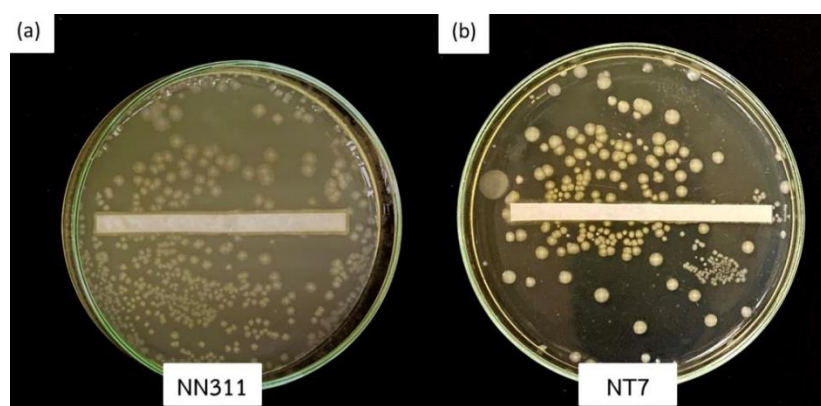


Figure 2. Microbial antagonism assay between the two selected isolates. The absence of inhibition zones confirms a non-antagonistic interaction. (a) Inhibition test of isolate NT7 against NN311 on paper strip, (b) Inhibition test of isolate NN311 against NT7 on paper strip

The absence of inhibitory effects between NT7 and NN311 suggests that their metabolic activities do not impede each other, potentially owing to niche differentiation or complementary resource utilization [28]. This compatibility is particularly advantageous in rhizosphere environments, where microbial cooperation can improve nutrient availability, such as through nitrogen fixation by *Bacillus* and phosphate solubilization by *Pseudomonas*, without causing cross-inhibition [29]. The lack of antagonism enhances their potential for use in biofertilizer formulations, as microbial synergy can more effectively enhance plant performance than single-strain inoculants [30].

3.4 Influence of Microorganisms on Oil Palm Growth

3.4.1 Physical Growth

The study used pre-germinated oil palm seeds with emerging shoots (1.0-1.5 cm) and roots (2.0-2.5 cm) to evaluate the effects of microbes on growth promotion. Seedlings received weekly microbial treatments for 5 months before physical growth parameters were measured, including fresh/dry weights (whole plant, shoot, root), leaf length, and leaf count. The results, although derived from different functional mechanisms, indicated that *Bacillus* sp. NT7, a nitrogen fixer, and *Pseudomonas* sp. NN311, a phosphate solubilizer, significantly enhances the growth of oil palm seedlings (Figure. 3, Table 4). The notable increase in root biomass (5.84 g vs. 5.01 g in NT7) was likely due to organic acids facilitating phosphorus mobilization, which in turn encourages root growth [31]. Conversely, NT7 enhanced leaf elongation (38.43 cm vs. 36.45 cm in NN311), which is consistent with its function in the assimilation of nitrogen for the development of shoots [32]. This functional complementarity demonstrates the benefits of consortia-based biofertilizers, as the simultaneous supply of phosphorus and nitrogen can mitigate single-nutrient deficiencies in tropical soils [33]. The discovery demonstrated the functional complementarity between nitrogen-fixing and phosphate-solubilizing microorganisms, indicating that their combined application provides greater growth benefits than inoculations of individual strains. The results suggested that using microbial consortia could be an effective biofertilizer strategy for oil palm cultivation.



Figure 3. Physical growth characteristics of oil palm seedlings under different treatments: (a) Control (untreated) seedlings, (b) Seedlings inoculated with NT7, (c) Seedlings inoculated with NN311, and (d) Seedlings co-inoculated with NN311+NT7.

3.4.2 Chlorophyll and Carotenoid Content

Chlorophyll is a crucial component of plant photosynthesis, working synergistically with carotenoids. Carotenoids absorb light for photosynthesis and shield chlorophyll from photodamage in high-light conditions [34], while chlorophyll transforms light energy into chemical energy to synthesize organic compounds, especially sugars, which act as precursors for numerous other organic molecules [35]. The molecular structure of chlorophyll includes nitrogen, enabling sufficient nitrogen availability, which is crucial for optimal plant growth [36]. After 5 months of growth, the levels of carotenoids and chlorophyll in the leaves were assessed to evaluate the effects of plant growth-promoting microorganisms on oil palm seedlings. The increased levels of chlorophyll and carotenoids in microbially treated oil palm seedlings highlighted the essential role of plant growth-promoting microorganisms in photosynthetic efficiency. The findings indicated that seedlings exposed to growth-promoting microorganisms showed markedly elevated leaf chlorophyll and carotenoid concentrations compared with the control group (Table 5). Significantly, the application of NT7, whether used independently or in conjunction with NN311, resulted in higher pigment concentrations than NN311 treatment alone. The study indicated that nitrogen-fixing microorganisms (NT7) significantly enhance photosynthetic pigment production, mainly by increasing nitrogen availability for chlorophyll synthesis. The enhanced efficacy of the NT7+NN311 treatment suggested a possible synergistic interaction between nitrogen fixation and phosphate solubilization, thereby improving photosynthetic efficiency and photoprotective mechanisms in oil palm seedlings. This physiological enhancement is associated with observed improvements in plant growth metrics, underscoring the significance of microbial-mediated nutrient acquisition for optimal plant development. Enhancing biofertilizer formulations for oil palm cultivation requires a thorough understanding of the specific effects of both individual and combinatorial microbial treatments.

Table 4. Physical growth parameters of oil palm seedlings under different microbial treatments

Treatment	gross weight (g)	stem weight (g)	root weight (g)	root shoot ratio (wet)	leaf length (cm)	total leave	gross dry weight (g)	stem dry weight (g)	root dry weight (g)	root shoot ratio (dry)
Control	12.4±1.0a	7.2±0.7a	2.5±0.1a	0.36±0.02a	33.9±2.4a	5.2±0.2a	2.8±0.2a	1.9±0.2a	0.88±0.04a	0.46±0.02b
NN311	23.7±3.4c	13.6±1.7b	5.8±1.0b	0.42±0.03b	36.4±2.9b	6.0±0.0b	5.3±0.8c	3.4±0.3c	1.89±0.47c	0.54±0.78c
NT7	21.5±0.8b	12.1±0.4b	5.0±0.2b	0.41±0.02b	38.4±1.4bc	5.5±0.03a	4.3±0.1b	2.9±0.1b	1.41±0.04b	0.48±0.04b
NN311+ NT7	24.0±0.8c	15.0±0.5c	5.4±0.6b	0.36±0.04a	41.8±1.0c	6.0±0.0b	5.0±0.3c	3.7±0.2c	1.37±0.13b	0.37±0.03a

Data represent mean ± SE. Means followed by the same lowercase letter do not differ significantly (Tukey's HSD test, $p \leq 0.05$).

Table 5. Chlorophyll and carotenoid content in oil palm (*E. guineensis* Jacq.) seedling leaves

Treatment	Chlorophyll a ($\mu\text{g cm}^{-2}$)	Chlorophyll b ($\mu\text{g cm}^{-2}$)	Total Chlorophyll ($\mu\text{g cm}^{-2}$)	Carotenoid ($\mu\text{g cm}^{-2}$)
Control	0.22 ± 0.01a	0.09 ± 0.00a	0.30 ± 0.01a	0.06 ± 0.00a
NN311	0.31 ± 0.00b	0.13 ± 0.00b	0.42 ± 0.01b	0.09 ± 0.00b
NT7	0.36 ± 0.02c	0.16 ± 0.01c	0.50 ± 0.03c	0.10 ± 0.01b
NN311+ NT7	0.40 ± 0.00c	0.18 ± 0.00c	0.55 ± 0.01c	0.12 ± 0.00c

Data represent mean ± SE. Means followed by the same lowercase letter do not differ significantly (Tukey's HSD test, $p \leq 0.05$).

3.4.3 Effects of Microorganisms on Oil Palm Growth After Field Transplantation

The field study revealed that the co-inoculation of *Pseudomonas* sp. NN311 and *Bacillus* sp. NT7 markedly enhanced oil palm establishment in clay soils. Treated seedlings exhibited a height increase of 70.9% (3.1 m compared to 1.8 m) and displayed enhanced stem morphology in comparison to the control group (Table 6, Figure 4 and 5). This growth enhancement resulted from synergistic nutrient cycling: NN311's organic acid-mediated phosphate solubilization augmented rock phosphate availability (from 500 g applications), while NT7's nitrogen fixation mitigated the typically low nitrogen retention in clay soils [37]. The 4.8 ha scale results validate laboratory findings and address a significant agricultural issue: clay soils impede root growth and nutrient absorption [38]. The treatment's efficacy (about 2 years of normal growth in 18 months) suggested the possibility of reducing the unproductive juvenile phase in oil palm farming, which often lasts 3-4 years [39]. The enduring effects suggested the ecological resilience of introduced strains, possibly via biofilm formation in the manure-amended rhizosphere [40]. This research provides empirical evidence that microbial consortia can serve as a sustainable alternative to chemical fertilizers in perennial crop systems, especially in clay soils.



Figure 4. Oil palm growth performance 18 months after field transplantation with microbial-augmented planting material.



Figure 5. Oil palm growth performance 18 months after field transplantation without microbial-augmented planting material.

Table 6. Oil palm growth performance 18 months after field transplantation with and without microbial-augmented planting material

Treatment	Height (m)	Stem girth (cm)	Number of fronds
Control	1.8 ± 0.2a	38.2 ± 0.02a	13.8 ± 1.0a
NN311+ NT7	3.1 ± 0.3b	66.7 ± 0.02b	33.8 ± 1.0b

Data represent mean ± SE. Means (same column) followed by the same lowercase letter do not differ significantly (ANOVA, $p \leq 0.05$).

4. Conclusions

This research isolated and characterized two efficacious microbial strains for oil palm cultivation: NN311 (*Pseudomonas* sp.), distinguished by its phosphate-solubilizing properties, and NT7 (*Bacillus* sp.), acknowledged for its nitrogen-fixing capabilities. Among the microbial colonies analyzed, NN311 and NT7 demonstrated the most substantial performance in both qualitative and quantitative assessments. These strains demonstrated no antagonistic effects when co-inoculated, enabling their combined application. Field tests demonstrated that both individual and combined applications of NN311 and NT7 significantly enhanced oil palm seedling growth compared to the control group, with the combined treatment yielding the most favorable results. After 18 months, the inoculated seedlings exhibited significantly superior growth compared to the untreated seedlings when these microorganisms were applied in agricultural fields alongside rock phosphate-enriched planting material. This research demonstrates that the simultaneous application of phosphate-solubilizing (*Pseudomonas* sp.) and nitrogen-fixing (*Bacillus* sp.) microorganisms constitutes an effective biological approach for improving the establishment and growth of oil palm seedlings. This microbial consortium offers a sustainable method to enhance nutrient availability and reduce dependence on chemical fertilizers in oil palm cultivation.

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