



Exploration of Patchouli (*Pogostemon cablin* Benth.) Indigenous Rhizosphere Bacteria as Biofertilizer Candidates in Dissolving Phosphate and Producing Growth Regulator

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Abstract

Patchouli is a plant that has high economic value due to its extensive application in many different industries. Despite its economic importance, the production of Patchouli has struggled to meet the demands of these industries. Addressing this challenge, one potential approach involves harnessing the capabilities of indigenous rhizosphere bacteria to enhance Patchouli growth. This study aims to investigate indigenous rhizosphere bacteria associated with Patchouli, focusing on their ability to solubilize phosphate and produce indole-3-acetic acid (IAA) and gibberellins. The research commenced with the isolation of microbes, which were subsequently evaluated for their phosphate-solubilizing and growth regulator-producing capacities. The results revealed the selection of ten isolates using Pikovskaya media, characterized by morphological variations, with 60% identified as gram-negative bacteria. Notably, three isolates (N1R1, N1R3, N1R7) exhibited superior phosphate-solubilizing abilities, with the highest average clear zone diameter recorded at 10.0 mm. Moreover, isolate N1R1 demonstrated the highest IAA production at 73.03 µg/ml, while isolate N1R7 exhibited the most substantial gibberellin (GA3) production at 98.18 µg/ml. Molecular identification indicated that N1R1 resembled *Providencia rettgeri*, N1R3 resembled *Enterobacter* sp., and N1R7 resembled *Erwinia* sp. In conclusion, three indigenous rhizosphere bacteria associated with Patchouli exhibit promising potential for biofertilizer development, given their proficiency in phosphate solubilization and growth regulator production.

Keywords: Rhizosphere, Patchouli, Phosphate, IAA, Gibberellin

1. Introduction

Patchouli (*Pogostemon cablin* Benth.) is a plant extensively utilized in diverse industries, including cosmetics, medicine, and as a raw material for perfumes and other fragrance-related sectors [1]. Patchouli oil can be derived from various plant parts, including leaves, roots, and stems, but the highest concentration is notably found in the dried leaves [2,3]. The quality of Patchouli oil is primarily contingent upon the content of Patchouli alcohol that can bind aromatic compounds from other sources [4,5]. Due to its unique properties, Patchouli alcohol cannot be effectively substituted with synthetic compounds [4]. Consequently, the utilization of Patchouli oil must be supported by the high productivity of the plant itself. Indonesia stands as the largest producer of Patchouli oil, followed by several other Asian countries [1]. However, Indonesian Patchouli farmers grapple with a common challenge—the insufficient productivity of Patchouli, hindering its capacity to meet the escalating demands for Patchouli oil in various industries. Consequently, there is a pressing need to enhance Patchouli growth productivity to adequately supply the demand for Patchouli oil. Farmers have sought to address this challenge by resorting to the application of synthetic or chemical fertilizers. However, the continuous use of such fertilizers

poses a threat to soil health. The prolonged application of chemical fertilizers contributes to soil acidification, which, in turn, hinders the growth of soil microbes, particularly rhizosphere bacteria [6]. This detrimental impact eventually leads to soil damage, characterized by a reduction in soil pore size and compromised soil aeration. Continuous reliance on chemical fertilizers poses a risk to the long-term sustainability of Patchouli cultivation and necessitates a more sustainable approach to enhance plant productivity without compromising soil health.

Numerous studies have investigated the efficacy of biofertilizers to restore soil conditions and enhance the rhizosphere microbial ecosystem. Within the soil, a diverse array of rhizosphere microbial communities exists, some of which play pivotal roles in plant growth promotion and disease suppression. Furthermore, these rhizosphere microbes demonstrate the ability to impede the proliferation of pathogenic bacteria through the synthesis of antimicrobial compounds [7,8]. The careful selection of rhizosphere microbes is essential to identify specific types that can effectively enhance the growth of Patchouli plants. Several studies have concentrated on the isolation and characterization of indigenous Plant Growth-Promoting Rhizobacteria (PGPR) from the rhizosphere of various plants, such as sugarcane [9] and mangroves [10]. These isolated bacteria have exhibited PGPR capabilities, promoting plant growth through mechanisms such as increased nutrient uptake, production of growth regulators, and suppression of plant pathogens [11]. A similar approach can be applied to Patchouli by exploring indigenous rhizosphere microbes that contribute to Patchouli growth through activities such as nutrient uptake and growth regulator production. This tailored selection of rhizosphere microbes holds the potential to significantly enhance the overall productivity and health of Patchouli cultivation.

One of the nutrients that is really needed to increase the growth production of patchouli leaves is phosphate, representing one of the macronutrients required in substantial quantities. However, in soil, phosphate availability is often constrained as it is bound to other compounds. Two predominant forms of phosphate exist in soil—organic and inorganic. Inorganic phosphate typically associates with Fe, Al, and Ca, while organic phosphate forms esters such as phospholipids, binds to C, or exists in the form of adenosine triphosphate and adenosine diphosphate [12]. Consequently, bacteria play a pivotal role in solubilizing phosphate, rendering it available for optimal absorption by plants. Bacterial genera such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Acinetobacter*, *Rhizobium*, and *Burkholderia* have demonstrated proficiency in phosphate dissolution [13]. Moreover, microbes also contribute to plant growth by synthesizing growth regulators such as Indole acetic acid (IAA) and gibberellin. Hence, the objective of this research is to systematically explore and collect indigenous rhizosphere bacteria candidates possessing the dual capacity to dissolve phosphate and produce growth regulators. These identified bacteria candidates hold promising potential for utilization in the development of biofertilizers. By leveraging microbial-based biofertilizers, there exists an opportunity to mitigate reliance on chemical fertilizers, not only enhancing plant productivity but also preserving soil fertility. This sustainable approach aligns with the broader goal of minimizing environmental impact and promoting soil health in Patchouli cultivation.

2. Materials and methods

2.1 Isolation of indigenous rhizosphere bacteria

Rhizosphere bacteria were isolated from the surface of Patchouli roots using a modified version of the method outlined by [14]. Sample was collecting from Saloadak village, Tobadak District, Central Mamuju Regency, West Sulawesi Province. The process involved the separation of Patchouli roots from the stem using a knife. Subsequently, the roots were immersed in 10 ml of sterile water and subjected to vortexing for 60 seconds. The water containing soil particles from the Patchouli roots was then extracted (1 ml) and combined with 9 ml of sterile water, followed by another round of vortexing. This resulted in a 10^{-1} serial dilution. The same procedure was repeated to achieve dilutions of 10^{-2} through 10^{-6} . Aliquots (20 μ l) from the 10^{-4} , 10^{-5} , and 10^{-6} dilutions were then inoculated onto Nutrient agar media (Himedia, India) and incubated for 24 hours. Following incubation, a selection of colonies was made, and these were subsequently transferred to Pikovskaya media (Himedia, India), a selective medium. The transfer was followed by another 24-hour incubation period at room temperature. This sequential isolation and cultivation process aimed to enrich for rhizosphere bacteria from the Patchouli roots, facilitating their subsequent characterization and assessment of their phosphate solubilization capabilities.

2.2 Morphological characterization of bacteria

Bacteria exhibiting growth on Pikovskaya media (Himedia, India) are indicative of their capacity to dissolve phosphate, marking them as candidates for subsequent testing. Morphological characterization of these bacteria involved a detailed examination of colony features, encompassing observations of colony shape, elevation, edges, and color.

2.3 Gram stain

Gram staining is a fundamental microbiological technique employed to ascertain the morphological characteristics of bacterial cells and to differentiate between gram-positive and gram-negative bacteria. This staining procedure relies on the utilization of a staining reagent kit, in this case, provided by Himedia, India.

2.4 Phosphate dissolving ability test

The selected isolates were cultured on Pikovskaya medium (Himedia, India) supplemented with phosphate sources, such as calcium phosphate and potassium phosphate, each at a final concentration of 5g/L. To standardize the pH of the medium, 1 N NaOH was added until reaching a pH of 7.0. The bacterial cultures were then incubated at 28°C for a duration of 48 hours. Subsequent to the incubation period, the medium was examined for the presence of a clear zone around the bacterial colonies. The appearance of such a clear zone is indicative of phosphate solubility [15]. The phosphate solubility index (IP) for each isolate can be calculated using the following formula [16]:

$$IP = \frac{\text{clear zone diameter} - \text{bacterial colony diameter}}{\text{bacterial colony diameter}} \quad (1)$$

2.5 IAA and Gibberellin production ability test

The assessment of bacterial isolates for their ability to produce Indole-3-acetic acid (IAA) involved cultivating the isolates on Nutrient broth media (Himedia, India) supplemented with 200 ppm tryptophan. The incubation of isolates occurred over a period of 6 days at 28°C. Following the incubation period, 10 ml of the culture medium was centrifuged at 6000 rpm for 10 minutes. Subsequently, 1 ml of the supernatant was transferred to a test tube covered with aluminum foil, and 4 ml of Salkowski reagent (Merck, America) was added. The mixture was then incubated in the dark for 30 minutes, after which absorbance was measured using a spectrophotometer at a wavelength of 535 nm [16]. For evaluating the production of gibberellins, bacterial isolates were grown in Nutrient broth media (Himedia, India) and incubated for 7 days. Following incubation, 10 ml of the culture filtrate was centrifuged at 8000 rpm for 10 minutes. Subsequently, 5 ml of the supernatant was treated with 30% HCl and incubated for 75 minutes at 28°C. The resulting supernatant was then used for absorbance observations using a spectrophotometer at a wavelength of 254 nm [17].

2.6 Bacterial Genome Isolation and Molecular Identification using 16S rRNA

The selected isolates were initially cultured on Nutrient Broth (NB) media and incubated for 24 hours. Following incubation, bacterial cells were harvested for the subsequent isolation of genomic DNA. DNA extraction was conducted in accordance with the procedures outlined in the genome purification kit provided by Promega. The extracted DNA was then subjected to amplification using the Polymerase Chain Reaction (PCR) method [18]. The PCR reaction mixture comprised 12.5 µl of Go Taq Green Master Mix PCR, 8.5 µl of ddH₂O, 1 µl of forward primer, 1 µl of reverse primer, and 2 µl of DNA template. Universal primers, namely primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'), were employed. The PCR cycling protocol involved an initial denaturation step at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and a final extension step at 72°C for 3 minutes. Subsequently, the amplified microbial DNA underwent electrophoresis at 110 V for 50 minutes. Electrophoresis results were visualized using a UV transilluminator. The PCR products were sent to FirstBase (Malaysia) for sequencing. Sequence alignment of the forward and reverse sequences was conducted using Bioedit software, while the sequencing results were analyzed using the DNA databases available on the National Center for Biotechnology Information (NCBI) site. The obtained sequencing data was used to construct a phylogenetic tree using Mega 11 software. This comprehensive molecular analysis facilitated the taxonomic classification of the isolated rhizosphere bacteria.

2.7 Data Analysis

Statistical analysis for assess the significant differences of phosphate solubility index was Analysis of Variance (ANOVA). Subsequently, Duncan Multiple Range Test was utilized to determine significant variations among the different treatments, with a significance level set at $p \geq 0.05$.

3. Results and Discussion

3.1 Morphological Characteristics and Gram Staining of Patchouli Rhizosphere Bacteria

The outcomes of bacterial isolation on Nutrient Agar (NA) media yielded numerous isolates, subsequently transferred to Piskovskaya media for the purpose of selecting isolates demonstrating phosphate solubilization capabilities. Those isolates thriving on Piskovskaya media were further scrutinized based on similarities in morphological characteristics, culminating in the identification of 10 isolates, as delineated in Table 1. Examination of Table 1 reveals a diversity in shapes, elevations, margins, and colony colors among the isolated phosphate-solubilizing bacteria. Predominantly, the colonies exhibited a circular shape with convex elevations, and an undulate margin. The consistent milky-white coloration of all isolates' colonies was noteworthy. Morphological traits serve as foundational criteria for bacterial identification and categorization. While each bacterial type manifests a unique colony shape, reliance on morphological observations for definitive identification necessitates corroborative data, given the inherent challenges in distinguishing bacterial colonies based solely on morphological features. As underscored by Sousa et al [19], the morphological diversity of bacterial colonies poses both intriguing and challenging aspects, as colonies of the same bacterial type may exhibit variations influenced by factors such as media type, incubation duration, growth methodology, and other pertinent variables.

Table 1 Morphological Characteristics of Rhizosphere Bacterial Colonies from Patchouli Plants

Isolates Code	Shape	Elevation	Margin	Colour
N1R1	Circular	Pulvinate	Undulate	Milky white
N1R2	Circular	Convex	Undulate	Milky white
N1R3	Circular	Convex	Undulate	Milky white
N1R6	Circular	Raised	Entire	Milky white
N1R7	Circular	Convex	Undulate	Milky white
N1R10	Circular	Convex	Entire	Milky white
N1R11	Circular	Raised	Entire	Milky white
N1R12	Irregular	Convex	Undulate	Milky white
N1R13	Circular	Convex	Entire	Milky white
N1A1	Irregular	Convex	Undulate	Milky white

In conjunction with the morphological assessment of bacterial colonies, Gram staining was employed to scrutinize the characteristics of individual bacterial cells, and the corresponding outcomes are detailed in Table 2. Gram staining procedures were executed after the purification of isolates to ensure the acquisition of a single colony. The results of Gram staining on indigenous rhizosphere bacterial isolates derived from Patchouli reveal a diversity in cell shapes, encompassing bacilli, cocci, and streptococci. Notably, sixty percent (60%) of the ten phosphate-solubilizing bacterial isolates exhibited a gram-negative staining pattern. This observation signifies that the predominant proportion of phosphate-solubilizing rhizosphere bacteria isolated from Patchouli belonged to the gram-negative bacterial category. The chromatic outcomes of the Gram staining process are visually represented in Figure 1 below.

Table 2 Gram Staining of Phosphate Solubilizing Bacteria from the rhizosphere of Patchouli Plants

Isolates code	Cell shape	Colour	Gram +/-
N1R1	Bacilli	Purple	Positive
N1R2	Coccus	Pink	Negative
N1R3	Coccus	Pink	Negative
N1R6	Coccus	Pink	Negative
N1R7	Coccus	Purple	Positive
N1R10	Bacilli	Purple	Positive
N1R11	Bacilli	Pink	Negative
N1R12	Coccus	Pink	Negative
N1R13	Streptococcus	Pink	Negative
N1A1	Bacilli	Purple	Positive

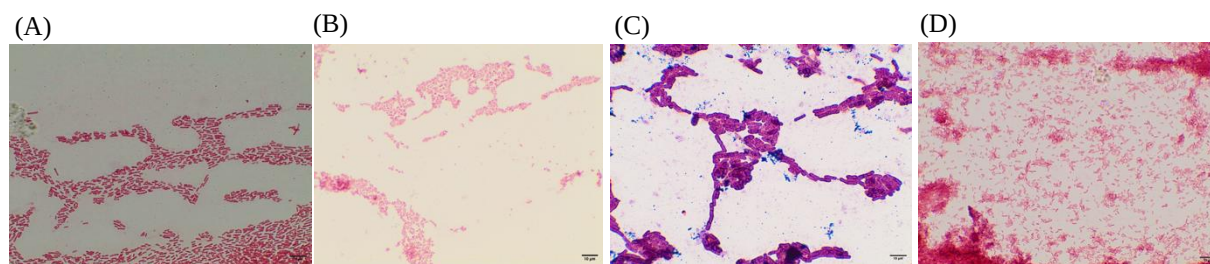


Figure 1 Results of gram staining of phosphate solubilizing bacteria a) N1R1; b) N1R3; c) N1A1; d) N1R1

3.2 Phosphate-dissolving ability

Phosphate-solubilizing bacteria possess the capability to facilitate the dissolution of phosphate bound to other compounds, rendering it accessible for plant absorption. The assessment of bacterial phosphate solubility is typically conducted by observing the formation of a clear zone in media supplemented with a phosphate source. Table 3 provides a comprehensive account of the phosphate solubilizing proficiency exhibited by 10 bacterial isolates. Notably, isolates N1R6 and N1A1 failed to produce any discernible clear zone, indicating a lack of phosphate-dissolving ability or an exceedingly weak solubilization capability, rendering the formed clear zone barely perceptible. Additionally, certain isolates, such as N1R2 and N1R10, exhibited clear zone diameters averaging lower than the bacterial colony's average diameter. This discrepancy arises from repeated treatments of several colonies, which failed to yield clear zones, resulting in negative phosphate solubility indices (PI). Comparable findings were reported by Sadiq et al [20], where several phosphate-dissolving bacterial isolates demonstrated a decline in phosphate-dissolving efficacy after multiple inoculation cycles. Consequently, among the ten isolates assessed, six demonstrated superior phosphate-dissolving abilities.

Table 3 The ability of rhizosphere bacteria from Patchouli to dissolve phosphate

Isolates Code	Average Diameter of Bacterial Colonies (mm)	Average Clear Zone Diameter (mm)	Phosphate Solubility Index (IP)
N1R1	5.8±0.12	10.0±0.06	0.74±0.02
N1R2	8.0±0.10	4.5±0.15	-0.44±0.01
N1R3	6.3±0.12	10.0±0.12	0.60±0.03
N1R6	7.0±0.06	0.0±0.00	-1.00±0.00
N1R7	5.8±0.15	10.0±0.06	0.74±0.04
N1R10	6.0±0.12	3.8±0.06	-0.38±0.00
N1R11	6.3±0.12	7.3±0.06	0.16±0.02
N1R12	5.8±0.06	7.3±0.10	0.27±0.03
N1R13	7.5±0.10	9.5±0.06	0.27±0.02
N1A1	5.3±0.06	0.0±0.00	-1.00±0.00

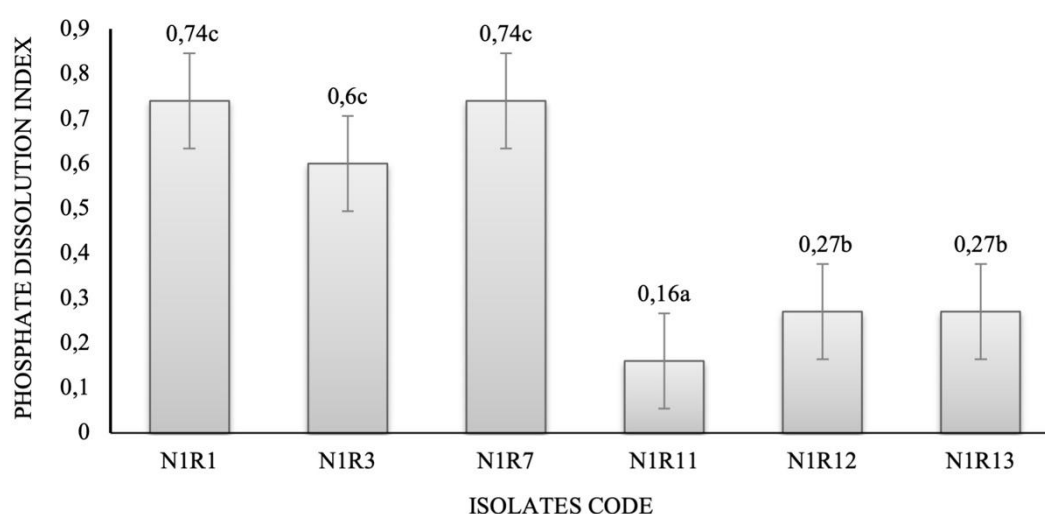


Figure 2 Results of ANOVA analysis of phosphate solubility index (IP) from several selected isolates

Note: Diagrams that have the same letter notation are not significantly different based on the DMRT test ($\alpha=0.05$)

Figure 2 illustrates the outcomes of the statistical analysis of phosphate solubilization ability (IP). The results indicate a significant disparity in phosphate-dissolving ability between isolate N1R11 with isolates N1R12, N1R13, N1R1, N1R3, and N1R7. Notably, isolates N1R1 and N1R7 exhibited the highest phosphate solubilization indices (IP), with no statistically significant difference observed compared to isolate N1R3. This underscores that these three isolates are highly recommended candidates for inclusion in a bacterial consortium for biofertilizer production, given their commendable capacity to dissolve phosphate. Phosphate solubilizing bacteria (PSB) play a crucial role in supporting plant growth, particularly in the context of macro-nutrient requirements, such as phosphate, essential for plants like Patchouli. Enhancing the productivity of Patchouli involves addressing phosphate nutrient availability. Phosphate solubilizing bacteria contribute by converting insoluble phosphate in the soil into a soluble form accessible for plant absorption [21]. Recognized genera of phosphate solubilizing bacteria encompass *Pseudomonas*, *Bacillus*, *Rhizobium*, *Micrococcus*, *Flavobacterium*, *Erwinia*, *Agrobacterium*, among others [20].

3.3 IAA and Gibberellin (GA3) Production Capability

The expectation was that phosphate-solubilizing bacteria would exhibit the additional capability of synthesizing growth regulators, specifically auxin (IAA) and gibberellin (GA3), to foster the growth of Patchouli plants. Evaluation for the production of growth regulators was conducted on the three most proficient isolates from the phosphate dissolution assay, alongside two additional isolates for comparative purposes (Table 4). The results mirrored those of the phosphate solubilization test, indicating that the three selected isolates (N1R1, N1R3, and N1R7) displayed higher concentrations of IAA and GA3 compared to the two comparison isolates (N1R11 and N1R13). Among the selected isolates, N1R1 exhibited the highest IAA concentration, reaching 73.03 $\mu\text{g/ml}$. This finding surpasses the IAA concentration produced by *Bacillus siamensis* isolated from peat, which ranged from 2.88 to 5.14 $\mu\text{g/ml}$ [22]. Mohite [23] suggests that variations in IAA production may be influenced by several factors such as bacterial type, strain, culture conditions, substrate, and growth phase. Meanwhile, isolate N1R7 demonstrated the highest GA3 concentration at 98.18 $\mu\text{g/ml}$. This result exceeds the GA3 concentration produced by rhizobacteria isolated from sugarcane roots, typically ranging from 20 to 24 $\mu\text{g/ml}$. Even after undergoing optimization through the modification of glucose and lactose sources, rhizobacteria achieved a GA3 concentration of 87.6 $\mu\text{g/ml}$ [24].

Table 4 Results of potential test for the production ability of growth regulators (IAA and GA3)

Isolates code	IAA concentrations ($\mu\text{g ml}^{-1}$)	GA3 concentrations ($\mu\text{g ml}^{-1}$)
N1R1	73.03	96.35
N1R3	40.58	97.45
N1R7	18.66	98.18
N1R11	14.55	95.49
N1R13	13.91	88.13

Three of ten bacterial isolates (N1R1, N1R3, and N1R7) derived from the rhizosphere of Patchouli exhibited the highest potential for both phosphate solubilization and growth regulator production, as indicated by the results of the potency tests conducted. These promising isolates stand out as potential candidates for inclusion in a bacterial consortium, which could be further developed into a biofertilizer product leveraging the indigenous bacteria associated with Patchouli plants. To ascertain their taxonomic identity, the molecular identification of these three bacterial isolates was undertaken. This molecular characterization aims to provide insight into the specific bacterial types present, contributing to a more comprehensive understanding of their potential application in biofertilizer development for Patchouli cultivation.

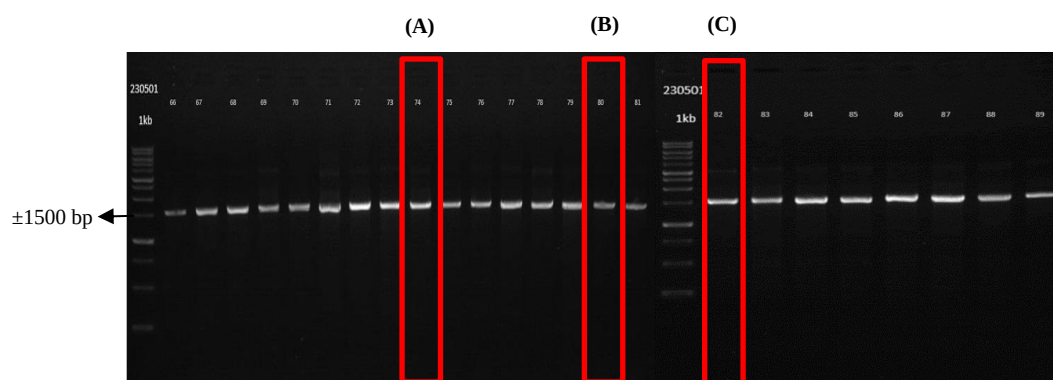


Figure 3 Agarose gel electrophoresis of polymerase chain reaction (PCR) amplification of indigenous rhizosphere bacteria DNA: a) N1R3; b) N1R7; dan c) N1R1

Table 5 The results of sequencing the indigenous rhizosphere bacteria of Patchouli which have the potential to dissolve phosphate and produce growth regulators

Isolate Code	Similarity (%)	Similar with species	Description	Accession Number
N1R1	88.83	<i>Providencia rettgeri</i>	<i>Providencia rettgeri</i> strain PR1 16S ribosomal RNA gene, partial sequence	KT985381.1
N1R3	88.43	<i>Enterobacter</i> sp.	<i>Enterobacter</i> sp. R2-2 16S ribosomal RNA gene, partial sequence	EU078565.1
N1R7	98.09	<i>Erwinia</i> sp.	<i>Erwinia</i> sp. strain CoA12 16S ribosomal RNA gene, partial sequence	MT557010.1

Figure 3 presents the electrophoresis results of DNA isolated from the three chosen isolates. The genomic products obtained from the PCR results were subsequently sequenced. The sequencing data underwent analysis utilizing information from NCBI, leading to identification results delineated in Table 5. The outcomes revealed that isolate N1R1 exhibited an 88.83% similarity to *Providencia rettgeri*, while N1R3 displayed an 88.43% similarity to *Enterobacter* sp. Furthermore, isolate N1R7 demonstrated a 98.09% similarity to *Erwinia* sp. The evolutionary relationship among these three isolates is elucidated in the phylogenetic tree depicted in Figure 4. Of the three isolates identified molecularly, have the similarity below 90% with isolates in database. This indicates the potential for these three isolates to be new strains, even new types. Therefore, further follow-up through whole genome sequencing analysis is needed.

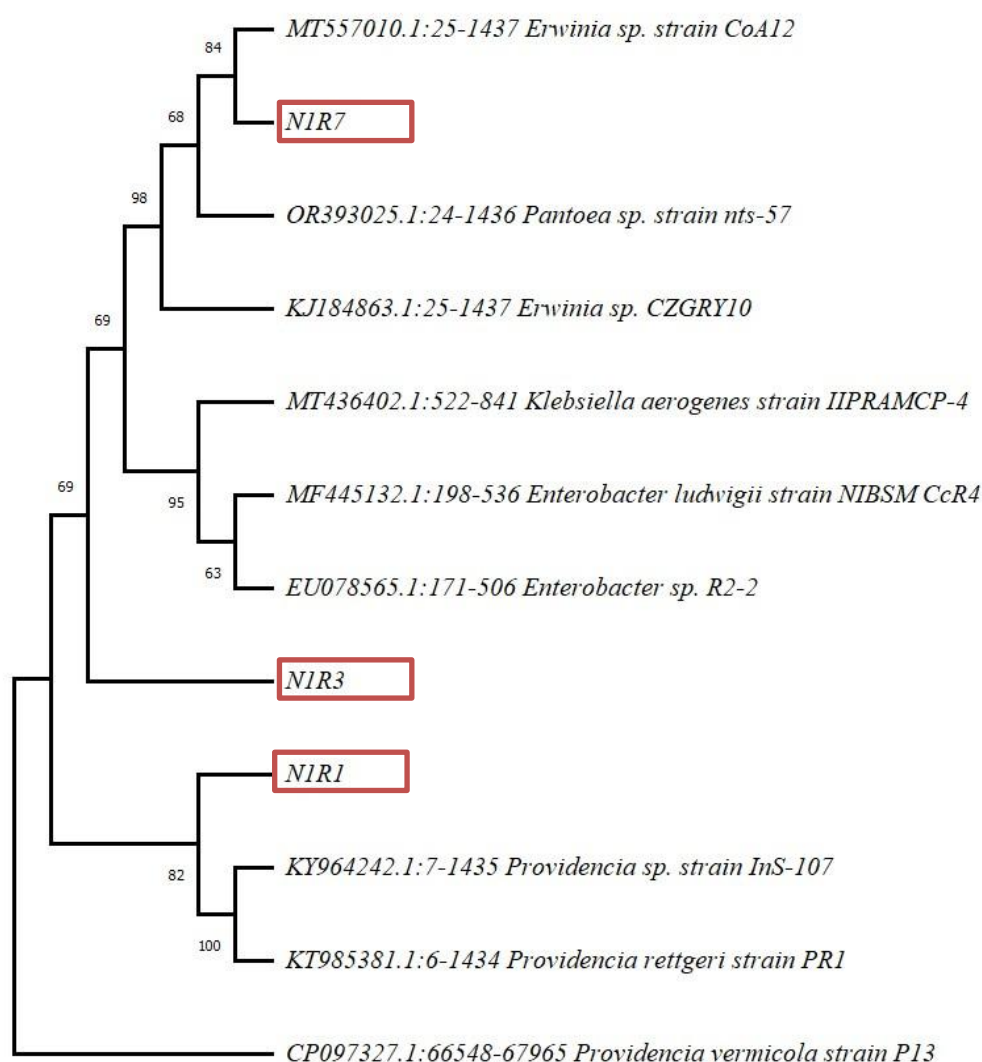


Figure 4 Phylogenetic tree of three bacterial isolates that can dissolve phosphate and produce growth regulator as biofertilizer candidates. Phylogenetic trees were constructed using the neighbor-joining (NJ) method with 1,000 bootstrap replications

Enterobacter sp. is a well-known type of bacteria acknowledged for its phosphate solubilization capability [25]. According to Jha et al [26], *Enterobacter* exhibits the potential to contribute to the development of sustainable agricultural systems. In general, *Enterobacter* serves agricultural ecosystems in three primary ways: synthesizing specific compounds beneficial for plants, facilitating the absorption of essential nutrients from the soil, and mitigating or preventing plant diseases. Gupta et al [27] conducted research highlighting that *Enterobacter* sp. CPSB49, in comparison to *Klebsiella* sp. CPSB4, demonstrated superior abilities in enhancing root length, shoot length, fresh and dry weight, and nutrient uptake in tomato plants. Furthermore, both bacteria were proven to aid in tomato growth under Chromium stress. *Erwinia* sp., akin to *Enterobacter*, is recognized for its beneficial impact on plant growth. Saldierna et al [28] conducted research demonstrating that *Erwinia gerundensis* A4, an endophytic bacterium isolated from almond plants, possesses phosphate solubilization abilities, evident from the clear zone surrounding the bacterial colony. Additionally, this bacterium has been proven to facilitate the growth of other plants, such as *Arabidopsis thaliana*, through root development. Regarding *Providencia* sp., it is acknowledged for its ability to produce indole-3-acetic acid (IAA). Research by Rushabh [29] highlighted that *Providencia* sp. (7MM11) under normal conditions, with a 24-hour incubation period, produced IAA with a concentration of 89.22 µg/ml. Although this concentration is less than the IAA concentration observed in this study, the variation could be attributed to different incubation times. In this study, isolates N1R1 was incubated for 6 days before assessing the IAA concentration. Rushabh's [29] research further explained that the concentration of IAA produced by *Providencia* sp. can be enhanced through various treatments, including the addition of L-tryptophan, increased incubation time, agitation of the incubation media, optimization of pH, and varying carbon sources. These findings collectively underscore the diverse and beneficial roles these bacterial isolates, specifically *Enterobacter* sp., *Erwinia* sp., and *Providencia* sp., could play in sustainable agriculture and plant growth promotion. Like *Enterobacter* sp., *Erwinia* sp., and *Providencia* sp., the three isolates observed also have good phosphate-dissolving and growth hormone-producing abilities. The combination of these two potentials will be beneficial for plants, supporting their growth by providing soluble phosphate, IAA, and gibberellin.

The comprehensive analysis presented above underscores the considerable potential of indigenous bacteria isolated from the rhizosphere of Patchouli to be developed into biofertilizers. This potential is particularly evident in their proficiency in phosphate solubilization and the synthesis of growth-regulating substances. Through the findings of this research, three isolates—N1R1, N1R3, and N1R7—have emerged as prime candidates for inclusion in a biofertilizer consortium. However, antagonistic testing among the three isolates should be conducted before developing a bacterial consortium to ensure the absence of inhibitory interactions between isolates. These selected isolates hold promise for fostering the growth of Patchouli plants, offering a sustainable approach to enhance plant productivity without adverse environmental impacts, thanks to the utilization of microbial-based fertilizers. This strategic application of indigenous bacteria in biofertilizer development aligns with the goal of optimizing Patchouli cultivation practices in an environmentally friendly manner. In the future, the potential of these three isolates is important to prove through direct application to patchouli plants.

4. Conclusion

10 isolates were identified as capable of thriving on selective media designed for phosphate-solubilizing bacteria. These bacterial isolates exhibited diverse morphological characteristics, with a notable predominance of gram-negative bacteria. Subsequent assessments revealed three isolates, namely N1R1, N1R3, and N1R7, demonstrated the highest phosphate solubility index values and capacity to produce growth regulators. Notably, N1R1 exhibited the highest concentration of IAA, while N1R7 showcased the highest gibberellin concentration. Consequently, these three isolates emerge as prime candidates for the development of biofertilizers. Molecular identification results unveiled that N1R1 bore similarity to *Providencia rettgeri*, N1R3 to *Enterobacter* sp., and N1R7 to *Erwinia* sp.

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6. Author Contribution

Conceptualization: APS, AA, BM; Data curation: APS, AA, NM, MW; Formal analysis: A, NA, MRTJPN; Funding acquisition: APS, AA; Methodology: BM, A, MW; Project administration: ADRAM, NA; Software: MRTJPN; Visualization: APS, NM; Writing- original draft: APS, AA; Writing- reviewing & editing: NM, MW.

7. Conflict of Interest

The authors declare no conflict of interest.

8. References

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