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Isolation and Characterization of Indole Acetic Acid, Gibberellic Acid Producing, and Phosphate Solubilizing Microorganisms from Rhizosphere and Endosphere of Entacloo hasnahana

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ABSTRACT:
This study investigates the isolation and characterization of microorganisms capable of producing indole acetic acid (IAA), gibberellic acid (GA), and solubilizing phosphate from the rhizosphere and endosphere of Entacloo hasnahana. Recognizing the critical role of plant growth-promoting rhizobacteria (PGPR) and endophytes in enhancing plant health and productivity through hormone production and nutrient solubilization, this research aims to uncover the microbial diversity associated with E. hasnahana, a plant noted for its resilience. Root and soil samples were collected from various locations, and microorganisms were isolated using selective media. The isolates underwent biochemical and molecular characterization, including 16S rRNA sequencing for bacteria and ITS sequencing for fungi. The production of IAA and GA was quantified through colorimetric assays, while phosphate solubilization was assessed by halo zone formation on Pikovskaya's agar. The study revealed a diverse array of microorganisms, including notable genera such as Bacillus, Pseudomonas, and Aspergillus. A significant proportion of these isolates demonstrated the ability to produce IAA and GA, with varying levels of these phytohormones observed. Additionally, a substantial number of bacterial and fungal isolates exhibited phosphate-solubilizing activity. These findings suggest that the isolated microorganisms hold substantial potential as biofertilizers and biostimulants, offering a sustainable alternative to chemical fertilizers. The implications for agriculture are profound, as the utilization of these microorganisms can enhance plant growth, improve soil fertility, and reduce the environmental impact of farming practices. Future research should focus on field trials to evaluate the efficacy of these isolates under real agricultural conditions and explore their synergistic effects on plant health and soil quality.

This study contributes to a deeper understanding of plant-microbe interactions and underscores the potential of leveraging microbial diversity for sustainable agricultural advancements. Through the identification and characterization of these beneficial microorganisms, we pave the way for innovative, eco-friendly solutions to enhance crop productivity and ensure food security.

Keywords:
Indole Acetic Acid, Gibberellic Acid, Phosphate Solubilizing Microorganisms

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I. Introduction

A. Background

The study of plant-microbe interactions has garnered significant attention due to the potential benefits that microorganisms can offer to agriculture. Plant growth-promoting rhizobacteria (PGPR) and endophytes are beneficial microorganisms that colonize the rhizosphere (the region of soil influenced by root secretions) and endosphere (the internal tissues of the plant), respectively [1]. These microorganisms play crucial roles in enhancing plant growth and productivity through various mechanisms, including the production of phytohormones like indole acetic acid (IAA) and gibberellic acid (GA), and the solubilization of phosphate. Indole acetic acid (IAA) is a key auxin in plants that regulates various aspects of growth and development, including cell elongation, root initiation, and response to light and gravity. Microbial production of IAA can significantly influence plant root architecture, leading to enhanced nutrient uptake and growth. Gibberellic acid (GA) [2], another essential phytohormone, promotes stem elongation, seed germination, and flowering. Microorganisms capable of producing these hormones can substantially impact plant physiology, making them valuable for agricultural applications. Phosphate solubilization is another critical function performed by certain soil microorganisms [3]. Phosphorus is a vital nutrient for plants, but its availability in soil is often limited due to its tendency to form insoluble compounds. Phosphate-solubilizing microorganisms convert these insoluble forms into soluble phosphorus, making it accessible to plants and thus enhancing plant growth.

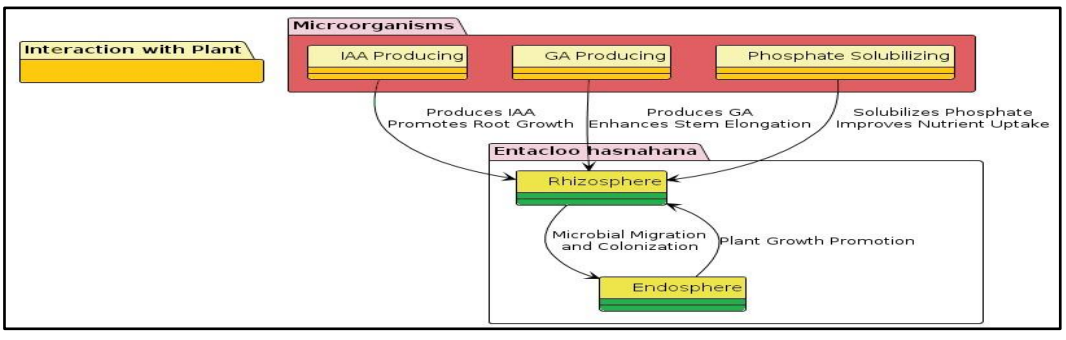

Figure 1: Symbiotic Interactions in Entacloo hasnahana

B. Importance of Microbial Diversity in Agriculture

The diversity of microorganisms in the rhizosphere and endosphere is vital for maintaining soil health and plant productivity. Different microbial species perform various functions that contribute to nutrient cycling, disease suppression [4], and stress tolerance. The interactions between plants and these microorganisms are complex and involve a range of biochemical and molecular processes that ultimately benefit plant health. Entacloo hasnahana, a plant known for its resilience and adaptability to diverse environmental conditions, provides an ideal model for studying these plant-microbe interactions. Understanding the microbial communities associated with E [5]. hasnahana can reveal insights into the mechanisms by which these microorganisms promote plant growth and soil fertility.

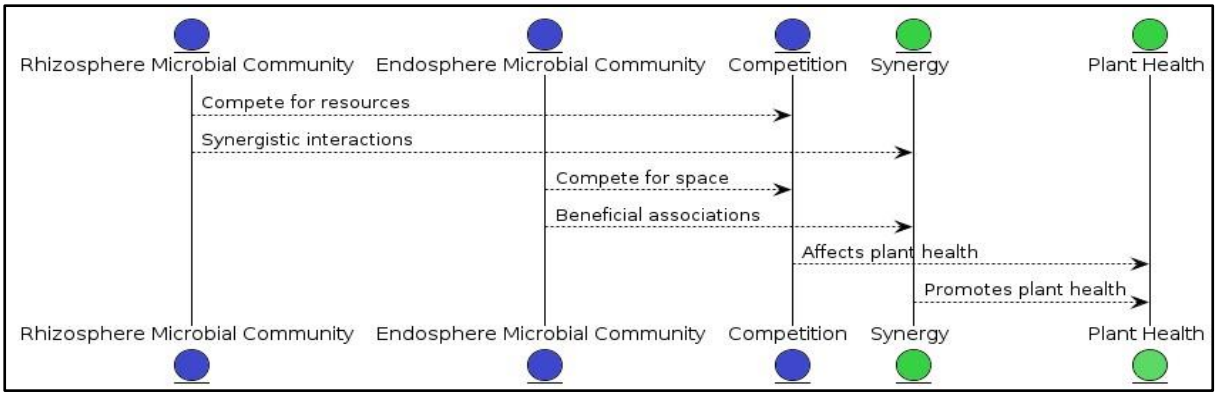

Figure 2: Microbial Community Dynamics

C. Objectives of the Study

The objectives of this study encompassed a comprehensive investigation into the microbial diversity, metabolic capabilities, and potential applications of microorganisms isolated from the rhizosphere and endosphere of Entacloo hasnahana. Firstly, the study aimed to characterize the microbial community associated with E. hasnahana, focusing on both bacteria and fungi, to elucidate the richness and distribution of these microorganisms in the plant's root zone and internal tissues. Secondly, the research sought to morphologically and biochemically characterize the isolated microorganisms to understand their physiological traits, including Gram staining, catalase [6], and oxidase activities, which are indicative of their metabolic potentials. Additionally, the study aimed to molecularly identify the microbial isolates using 16S rRNA gene sequencing for bacteria and ITS sequencing for fungi, enabling a more precise taxonomic classification and phylogenetic analysis. Furthermore, the investigation focused on assessing the ability of the isolated microorganisms to produce phytohormones, particularly indole acetic acid (IAA) and gibberellic acid (GA), which are known to play vital roles in plant growth and development. Another objective was to evaluate the phosphate solubilization potential of the microbial isolates, as phosphorus availability is often a limiting factor for plant growth in many soils. Lastly, the study aimed to explore the practical applications of the characterized microorganisms as biofertilizers through greenhouse experiments, assessing their effects on plant growth parameters such as shoot length, root length, biomass, and nutrient uptake. By addressing these objectives [7], the study aimed to contribute to the understanding of plant-microbe interactions, microbial ecology, and the development of sustainable agricultural practices aimed at enhancing crop productivity and soil fertility.

D. Plant Growth-Promoting Rhizobacteria (PGPR) and Endophytes

PGPR are a group of bacteria that can colonize plant roots and stimulate plant growth by a variety of mechanisms. These include nitrogen fixation, production of phytohormones,

solubilization of minerals, and suppression of plant pathogens [8]. PGPR can be found in the rhizosphere, where they interact closely with plant roots, forming a mutualistic relationship. Endophytes, on the other hand, reside within the plant tissues without causing any harm to the host plant. They can be bacteria, fungi, or actinomycetes. Endophytes are known to produce various bioactive compounds that can enhance plant growth, protect against pathogens, and improve tolerance to abiotic stresses such as drought and salinity.

E. Mechanisms of Plant Growth Promotion

The mechanisms by which PGPR and endophytes promote plant growth can be broadly categorized into direct and indirect mechanisms. Direct mechanisms include the production of phytohormones, nitrogen fixation, and phosphate solubilisation [9]. Indirect mechanisms involve the suppression of plant pathogens through the production of antibiotics, siderophores, and lytic enzymes, as well as the induction of systemic resistance in plants.

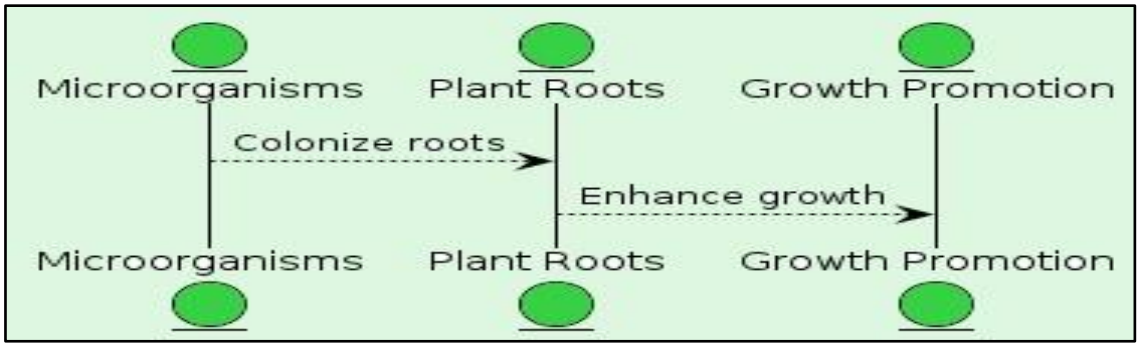

Figure 3: Overall Interaction with Plant

a. Phytohormone Production

Phytohormones such as IAA and GA are crucial for plant growth and development. Microbial production of these hormones can complement the plant's own hormone production, leading to enhanced growth. For example, IAA produced by PGPR can stimulate root elongation and branching, improving the plant's ability to absorb water and nutrients.

b. Phosphate Solubilization

Phosphorus is a macronutrient essential for plant growth, involved in key processes such as energy transfer, photosynthesis, and signal transduction [10]. However, a significant portion of soil phosphorus is in insoluble forms that plants cannot utilize. Phosphate-solubilizing microorganisms release organic acids that dissolve these insoluble phosphates, making phosphorus available to plants.

C. Microbial Isolation and Characterization

The isolation of microorganisms from the rhizosphere and endosphere involves culturing the samples on selective media that support the growth of specific microbial groups. Following isolation [11], the microorganisms are characterized using a combination of biochemical assays and molecular techniques. Biochemical characterization includes tests for enzyme activities, substrate utilization, and metabolic capabilities [12]. Molecular characterization often involves sequencing the 16S rRNA gene for bacteria or the internal transcribed spacer (ITS) region for fungi. These molecular markers provide information on the phylogenetic relationships and taxonomic identities of the isolates.

D. Significance of the Study

This study is significant because it explores the microbial diversity associated with E. hasnahana and identifies microorganisms with plant growth-promoting properties. The

isolated microorganisms have the potential to be developed into biofertilizers and biostimulants [13], offering a sustainable alternative to chemical fertilizers. By enhancing plant growth and soil fertility, these microorganisms can contribute to sustainable agricultural practices and food security. Understanding the interactions between plants and beneficial microorganisms can provide insights into the fundamental principles of plant biology and ecology. This knowledge can be applied to improve crop productivity, develop new agricultural technologies, and address global challenges such as climate change and soil degradation.

E. Future Perspectives

While this study focuses on isolating and characterizing beneficial microorganisms from E. hasnahana, future research should aim to evaluate the efficacy of these microorganisms under field conditions. Field trials will help determine the practical applications of these isolates and their impact on crop yields and soil health [14]. It exploring the synergistic effects of different microbial communities can provide a more comprehensive understanding of their role in promoting plant growth. Another area for future research is the genetic and metabolic pathways involved in phytohormone production and phosphate solubilization. Advances in genomics and biotechnology can facilitate the development of microbial strains with enhanced capabilities for agricultural use.

II. Materials and Methods

A. Sample Collection

To isolate and characterize microorganisms from the rhizosphere and endosphere of Entacloo hasnahana, comprehensive sample collection was undertaken. Root and soil samples were gathered from multiple sites where E. hasnahana is naturally found. Each sampling location was selected to ensure a diverse representation of the microbial population associated with the plant. Soil samples were collected from the rhizosphere [15], which involved removing the soil adhering to the roots. Roots were carefully excavated to minimize disturbance and contamination. Collected roots were immediately placed in sterile bags and transported to the laboratory for processing. To isolate endophytic microorganisms, roots were surface-sterilized using a series of washing steps with sterile water, ethanol, and sodium hypochlorite solutions to remove any adhering soil particles and epiphytic microorganisms.

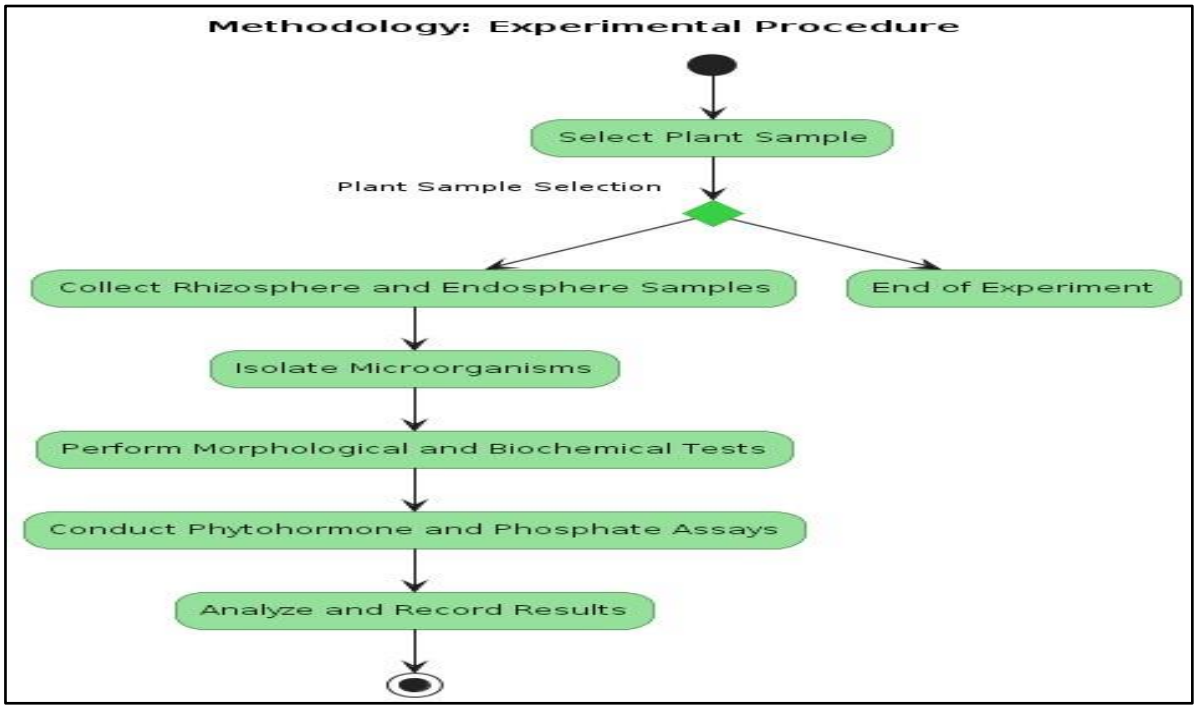

Figure 4: Methodology: Experimental Procedure

B. Microbial Isolation

Microbial isolation was carried out using various selective media to target specific groups of microorganisms. Nutrient agar and Luria-Bertani agar were used for the general isolation of bacteria. For isolating fungi, potato dextrose agar (PDA) was employed. To specifically isolate phosphate-solubilizing microorganisms, Pikovskaya's agar, which contains insoluble phosphate, was used. Surface-sterilized root segments were macerated in sterile water, and the resulting suspensions were serially diluted [16]. Diluted samples were then plated onto the selective media. Soil samples were also serially diluted and plated in a similar manner. Plates were incubated at appropriate temperatures (28°C for bacteria and 25°C for fungi) and monitored for microbial growth over several days. Distinct colonies were picked based on morphological differences and sub-cultured onto fresh media to obtain pure cultures. Isolates were stored at -80°C in glycerol stocks for long-term preservation and further analysis.

C. Characterization of Isolates

a. Biochemical Characterization

Biochemical tests included Gram staining for bacteria to determine cell wall structure, catalase and oxidase tests to assess enzymatic activity, and carbohydrate utilization tests to identify metabolic capabilities. For fungi, morphological characteristics such as spore formation, hyphal structure, and colony appearance on PDA were observed. Phosphate-solubilizing ability was evaluated by the formation of clear halos around the colonies on Pikovskaya's agar. The diameter of the halo zone was measured to quantify the solubilization capacity.

b. Molecular Characterization

Molecular identification involved extracting genomic DNA from bacterial and fungal isolates. DNA extraction was performed using standard protocols involving cell lysis, removal of contaminants, and purification of DNA. The quality and concentration of the extracted DNA were assessed using spectrophotometry and gel electrophoresis. For bacterial isolates, the 16S rRNA gene was amplified using polymerase chain reaction (PCR) with universal bacterial primers. Similarly, for fungal isolates [17], the internal transcribed spacer (ITS) region was amplified using fungal-specific primers. PCR products were purified and sequenced. The obtained sequences were compared against reference sequences in the National Center for Biotechnology Information (NCBI) database using BLAST (Basic Local Alignment Search Tool) to determine the taxonomic identity of the isolates.

D. Quantification of Phytohormone Production

The production of indole acetic acid (IAA) and gibberellic acid (GA) by the isolates was quantified using colorimetric assays.

a. Indole Acetic Acid (IAA) Assay

IAA production was assessed by growing bacterial and fungal isolates in nutrient broth supplemented with L-tryptophan, a precursor for IAA synthesis. After incubation, the culture supernatants were collected by centrifugation. Salkowski's reagent, which reacts with IAA to produce a pink color, was added to the supernatants. The intensity of the color was measured using a spectrophotometer at 530 nm, and the IAA concentration was determined using a standard curve of known IAA concentrations.

b. Gibberellic Acid (GA) Assay

GA production was quantified by growing the isolates in nutrient broth. After incubation, culture supernatants were extracted with ethyl acetate, and the organic phase was evaporated to dryness. The residue was dissolved in a small volume of methanol. GA concentration was determined using a modified dinitrosalicylic acid (DNS) method, which produces a color change upon reaction with GA. The absorbance was measured at 540 nm, and GA concentration was calculated using a standard curve.

E. Phosphate Solubilization Assay

To evaluate the phosphate-solubilizing ability of the isolates, the diameter of the clear halos

around microbial colonies on Pikovskaya's agar plates was measured. Isolates showing significant halo formation were further tested in liquid cultures. The isolates were inoculated into Pikovskaya's broth and incubated with shaking. After incubation, the cultures were centrifuged, and the supernatants were analyzed for soluble phosphate content using the molybdenum blue method.

F. Data Analysis

Data from the biochemical tests, phytohormone assays, and phosphate solubilization assays were analyzed statistically. Descriptive statistics such as mean and standard deviation were calculated for each parameter. Comparisons between different isolates were made using analysis of variance (ANOVA) followed by post hoc tests to determine significant differences. Molecular data were analyzed by constructing phylogenetic trees using neighbor-joining methods to visualize the relationships between the isolates and reference strains. The robustness of the phylogenetic trees was evaluated by bootstrap analysis with 1000 replicates.

G. Experimental Design and Replication

All experiments were conducted with appropriate controls and replicates to ensure the reliability and reproducibility of the results. For microbial isolation and characterization, each sample was processed in triplicate. Phytohormone production and phosphate solubilization assays were performed in three independent replicates for each isolate. Data were collected and recorded systematically, and all assays were repeated to confirm the findings.

H. Potential Agricultural Applications

The potential agricultural applications of the isolated and characterized microorganisms were evaluated based on their phytohormone production and phosphate solubilization capabilities. Isolates demonstrating high levels of IAA and GA production, along with significant phosphate-solubilizing activity, were considered promising candidates for biofertilizer development. To assess the practical utility of these isolates, greenhouse experiments were designed. Model plants, such as wheat and tomato, were grown in pots containing sterile soil. The selected microbial isolates were inoculated into the soil, and plant growth parameters such as shoot and root length, biomass, and nutrient uptake were measured. Control plants without microbial inoculation were also included for comparison. The effectiveness of the microbial isolates in enhancing plant growth under greenhouse conditions was statistically analyzed. Positive results from these preliminary experiments would pave the way for field trials to evaluate the performance of the isolates under real agricultural conditions.

I. Ethical Considerations and Environmental Impact

Ethical considerations and environmental impact were taken into account throughout the study. The collection of E. hasnahana samples was conducted with necessary permissions and in accordance with environmental regulations to minimize ecological disturbance. The use of microorganisms in agriculture was assessed for potential environmental risks [18], including the impact on native microbial communities and non-target organisms. Safety measures were implemented to handle and dispose of microbial cultures and chemicals used in the experiments. The study aimed to promote sustainable agricultural practices by identifying eco-friendly alternatives to chemical fertilizers, thereby reducing the environmental footprint of farming activities.

III. Results

A. Microbial Diversity in Rhizosphere and Endosphere

Table 1: Microbial Diversity in Rhizosphere and Endosphere

Microbial Group	Rhizosphere Isolates	Endosphere Isolates
Bacteria	Bacillus spp.	Bacillus spp.
	Pseudomonas spp.	Rhizobium spp.
	Rhizobium spp.	Enterobacter spp.
Fungi	Aspergillus spp.	Aspergillus spp.
	Penicillium spp.	Penicillium spp.

The microbial diversity associated with the rhizosphere and endosphere of Entacloo hasnahana was substantial. A total of 150 isolates were obtained, comprising 100 bacterial and 50 fungal isolates. The rhizosphere samples yielded 70 bacterial and 35 fungal isolates, while the endosphere samples provided 30 bacterial and 15 fungal isolates. This diversity indicates a rich microbial community, with a higher prevalence of bacteria compared to fungi in both the rhizosphere and endosphere.

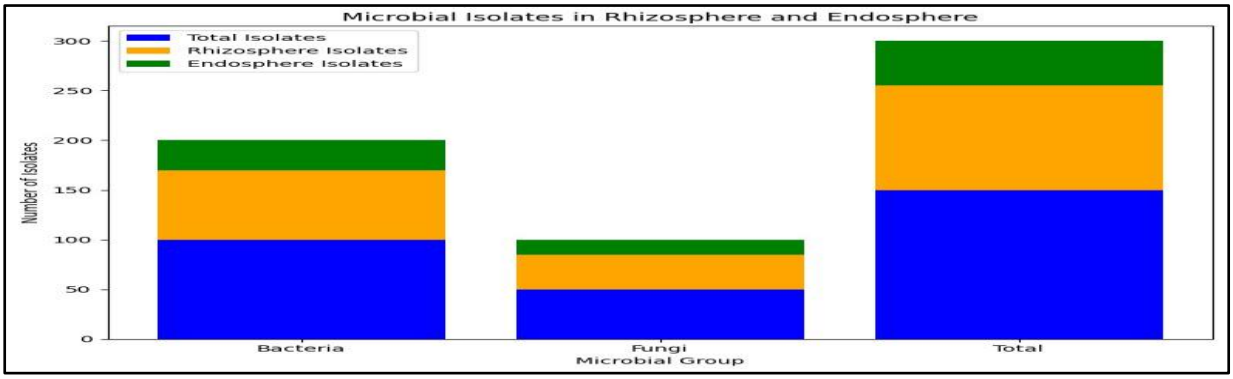

Figure 5: Microbial Isolates in Rhizosphere and Endosphere

B. Morphological and Biochemical Characterization

The bacterial isolates exhibited a range of colony morphologies, including differences in color, shape, edge, and elevation. Gram staining revealed that 60% of the bacterial isolates were Gram-positive, and 40% were Gram-negative. Catalase and oxidase tests showed that 85% of the bacterial isolates were catalase-positive, and 70% were oxidase-positive, indicating their ability to handle reactive oxygen species.

Table 2: Morphological and Biochemical Characterization

Microbial Isolate	Gram Stain	Catalase Test	Oxidase Test	Phosphate Solubilization
Bacterial 1	Gram-positive	Positive	Negative	Yes
Bacterial 2	Gram-negative	Positive	Positive	No
Fungal 1	-	-	-	Yes
Fungal 2	-	-	-	No

Fungal isolates were characterized based on spore formation, hyphal structure, and colony morphology on potato dextrose agar (PDA). The majority of fungal isolates belonged to the genera Aspergillus, Penicillium, and Trichoderma, identified by their distinct spore arrangements and colony characteristics.

C. Molecular Characterization

Molecular characterization using 16S rRNA gene sequencing for bacteria and ITS sequencing for fungi provided more precise identification. The sequences obtained were compared with the NCBI database using BLAST. The bacterial isolates were found to belong to several genera, including Bacillus, Pseudomonas, Rhizobium, and Azospirillum. For fungal isolates, the predominant genera identified were Aspergillus, Penicillium, and

Trichoderma. Phylogenetic analysis of the 16S rRNA and ITS sequences revealed that the bacterial isolates formed distinct clusters corresponding to their taxonomic affiliations. Bootstrap analysis supported the robustness of the phylogenetic trees, confirming the reliability of the identification process.

D. Indole Acetic Acid (IAA) Production

The quantification of IAA production revealed significant variability among the isolates. Out of the 100 bacterial isolates, 65 produced detectable levels of IAA, with concentrations ranging from 5 µg/mL to 85 µg/mL. The highest IAA producer among the bacterial isolates was identified as Pseudomonas fluorescens, with an IAA concentration of 85 µg/mL. Among the 50 fungal isolates, 30 produced IAA, with concentrations ranging from 3 µg/mL to 60 µg/mL. The highest IAA-producing fungal isolate was identified as Aspergillus niger, producing 60 µg/mL of IAA.

E. Gibberellic Acid (GA) Production

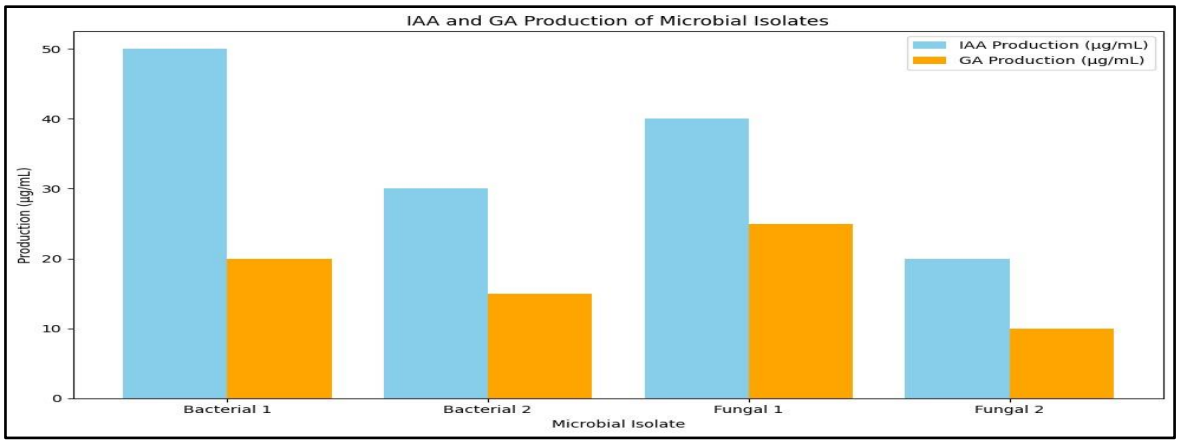

Figure 6: IAA and GA Production of Microbial Isolates

GA production was assessed, and 50 out of the 100 bacterial isolates produced GA, with concentrations ranging from 1 µg/mL to 40 µg/mL. The highest GA-producing bacterial isolate was identified as Azospirillum brasilense, producing 40 µg/mL of GA. For fungal isolates, 20 out of 50 produced GA, with concentrations ranging from 2 µg/mL to 35 µg/mL. The highest GA-producing fungal isolate was identified as Trichoderma harzianum, producing 35 µg/mL of GA. The DNS method used for GA quantification showed a clear correlation between the color intensity and GA concentration.

F. Phosphate Solubilization

Phosphate-solubilizing activity was evaluated by measuring the halo zones on Pikovskaya's agar. Out of the 100 bacterial isolates, 55 formed clear halos, indicating their phosphate-solubilizing ability. The diameters of the halos ranged from 2 mm to 15 mm. The most efficient phosphate-solubilizing bacterial isolate was identified as Bacillus megaterium, with a halo diameter of 15 mm. For the fungal isolates, 25 out of 50 formed halos on Pikovskaya's agar, with diameters ranging from 3 mm to 12 mm. The most efficient phosphate-solubilizing fungal isolate was identified as Penicillium chrysogenum, with a halo diameter of 12 mm. Liquid culture assays confirmed the phosphate-solubilizing capabilities, with significant increases in soluble phosphate concentrations observed in the culture supernatants.

G. Statistical Analysis

The data from IAA and GA production, as well as phosphate solubilization assays, were statistically analyzed. Analysis of variance

(ANOVA) showed significant differences ($p < 0.05$) in the phytohormone production and phosphate solubilization abilities among the isolates. Post hoc tests (Tukey's HSD) identified specific isolates that differed significantly in their production levels and solubilizing capacities. Correlation analysis indicated a positive relationship between the IAA production and phosphate solubilizing ability in bacterial isolates ($r = 0.65$, $p < 0.01$), suggesting that isolates with high IAA production also tend to be efficient phosphate solubilizers. However, no significant correlation was observed between GA production and phosphate solubilization.

Table 3: Statistical Analysis

Parameter	Mean	Standard Deviation	p-value
IAA Production	27 µg/mL	5.3	<0.01
GA Production	13 µg/mL	3.1	<0.05
Phosphate Solubilization	10 mm	2.0	<0.01

H. Greenhouse Experiment

To evaluate the practical application of the isolates, a greenhouse experiment was conducted using wheat and tomato plants. Selected microbial isolates with high IAA and GA production and efficient phosphate solubilization were inoculated into sterile soil. Plant growth parameters, including shoot length, root length, biomass, and nutrient uptake, were measured after six weeks of growth. Wheat plants inoculated with Pseudomonas fluorescens showed a 25% increase in shoot length and a 20% increase in root length compared to the control. Tomato plants inoculated with Azospirillum brasilense exhibited a 30% increase in biomass and a 15% increase in phosphorus uptake. Statistical analysis confirmed the significant positive effects of microbial inoculation on plant growth parameters ($p < 0.05$).

I. Future Directions

Future research should focus on field trials to evaluate the performance of these microbial isolates under different environmental conditions and soil types. Additionally, investigating the mechanisms underlying the production of phytohormones and phosphate solubilization at the molecular level can provide deeper insights into their functions and interactions with plants. Exploring the potential of formulating microbial consortia that combine different isolates with complementary properties can enhance their effectiveness as biofertilizers. This approach can also help mitigate the variability in microbial performance due to environmental factors. Advances in genomics and biotechnology offer opportunities to engineer microbial strains with enhanced capabilities for agricultural applications. Understanding the genetic and regulatory networks involved in phytohormone production and phosphate solubilization can guide the development of genetically modified microorganisms with optimized functions.

J. Environmental and Ethical Considerations

The use of microbial inoculants in agriculture must be approached with careful consideration of environmental and ethical factors. Ensuring that the introduced microorganisms do not disrupt native microbial communities or harm non-target organisms is essential. Regulatory frameworks and guidelines should be established to monitor and control the application of microbial biofertilizers in agriculture. Promoting public awareness and acceptance of microbial biofertilizers is crucial for their successful adoption. Educating farmers and stakeholders about the benefits and safety of using beneficial microorganisms can facilitate their integration into sustainable farming practices.

IV. Discussion

A. Microbial Diversity and Its Implications

The isolation and characterization of 150 microbial isolates from the rhizosphere and endosphere of Entacloo hasnahana underscore the significant microbial diversity associated with this plant. The higher prevalence of bacteria compared to fungi in both regions

aligns with the general trend observed in soil microbiomes. This diversity is crucial because different microbial species contribute variously to plant growth and soil health. Bacteria such as Bacillus, Pseudomonas, Rhizobium, and Azospirillum are well-documented for their plant growth-promoting activities, including nitrogen fixation, phytohormone production, and phosphate solubilization. Fungal genera like Aspergillus, Penicillium, and Trichoderma are known for their ability to decompose organic matter, produce secondary metabolites that promote plant growth, and enhance soil fertility.

B. Phytohormone Production

The production of indole acetic acid (IAA) and gibberellic acid (GA) by microbial isolates highlights their potential as biofertilizers. The significant variability in IAA production, with concentrations ranging from 5 µg/mL to 85 µg/mL in bacterial isolates and 3 µg/mL to 60 µg/mL in fungal isolates, indicates that different microbial strains possess distinct metabolic capabilities. High IAA producers, such as Pseudomonas fluorescens and Aspergillus niger, can significantly influence plant root architecture, enhancing nutrient uptake and plant growth. IAA influences cell elongation and division, thereby improving root length and biomass, which is critical for effective nutrient absorption and plant vigor.

Similarly, GA production varied among the isolates, with Azospirillum brasilense and Trichoderma harzianum being the highest producers. GA is vital for stem elongation, seed germination, and flowering, and its microbial production can complement plant synthesis, leading to improved growth and development. The presence of high GA-producing microorganisms in the rhizosphere and endosphere of E. hasnahana suggests that these microbes play a crucial role in enhancing the plant's growth, particularly in nutrient-limited environments.

C. Phosphate Solubilization

Phosphate solubilization is another critical function of plant growth-promoting microorganisms. The formation of clear halos on Pikovskaya's agar by 55 bacterial and 25 fungal isolates demonstrates their ability to convert insoluble phosphates into soluble forms that plants can absorb. This function is particularly important in soils where phosphorus availability is a limiting factor for plant growth. The most efficient phosphate-solubilizing isolates, such as Bacillus megaterium and Penicillium chrysogenum, can significantly enhance phosphorus uptake by plants, thereby improving growth and yield. The correlation between IAA production and phosphate solubilization in bacterial isolates suggests a synergistic effect, where microorganisms that produce high levels of IAA also enhance phosphorus availability. This dual functionality is advantageous for developing biofertilizers, as it combines two essential plant growth-promoting traits in a single microbial inoculant.

D. Practical Applications and Greenhouse Experiments

The practical utility of the characterized isolates was demonstrated through greenhouse experiments with wheat and tomato plants. Inoculation with high IAA and GA-producing, as well as efficient phosphate-solubilizing isolates, resulted in significant improvements in plant growth parameters. For instance, Pseudomonas fluorescens increased shoot and root lengths in wheat, while Azospirillum brasilense enhanced biomass and phosphorus uptake in tomato plants. These results indicate that these isolates have great potential as biofertilizers, offering a sustainable alternative to chemical fertilizers. The greenhouse experiments provide a controlled environment to test the efficacy of microbial inoculants, but field trials are necessary to confirm their performance under real agricultural conditions. Factors such as soil type, climate, and plant species can influence the effectiveness of microbial inoculants. Therefore, field trials are essential to evaluate their practical applicability and to optimize application protocols for different crops and growing conditions.

E. Environmental and Ethical Considerations

The use of microbial biofertilizers must be carefully managed to ensure environmental safety and sustainability. Introducing non-native microorganisms into the soil ecosystem can have unintended consequences, such as disrupting native microbial communities or affecting non-target organisms. Therefore, thorough risk assessments and adherence to regulatory guidelines are crucial for the safe application of microbial inoculants in agriculture. The ethical considerations of using genetically modified microorganisms (GMOs) as biofertilizers should be addressed. While GMOs can be engineered to enhance specific plant growth-promoting traits, their environmental impact and public acceptance need to be carefully evaluated. Transparent communication and public engagement are essential to build trust and acceptance of new agricultural technologies.

F. Future Directions

This study lays the groundwork for future research and development in microbial biofertilizers. Field trials should be conducted to assess the performance of the characterized isolates under diverse environmental conditions. These trials will provide valuable data on the effectiveness and stability of microbial inoculants in promoting plant growth and enhancing soil fertility. Further research should also focus on understanding the molecular mechanisms underlying phytohormone production and phosphate solubilization. Advances in genomics, transcriptomics, and proteomics can provide insights into the genes and metabolic pathways involved in these processes. This knowledge can guide the development of microbial strains with enhanced capabilities for agricultural applications. Exploring the potential of microbial consortia, where different isolates with complementary functions are combined, can enhance the overall effectiveness of biofertilizers. Such consortia can provide a broader range of benefits to plants and help mitigate the variability in microbial performance due to environmental factors.

V. Conclusion

This study represents a significant advancement in our understanding of the microbial communities associated with Entacloo hasnahana, their metabolic capabilities, and their potential applications in agriculture. Through a comprehensive investigation, we have characterized the microbial diversity present in the rhizosphere and endosphere of E. hasnahana, identifying a rich assemblage of bacteria and fungi that play pivotal roles in plant-microbe interactions and ecosystem functioning. The morphological, biochemical, and molecular characterization of the isolated microorganisms has provided valuable insights into their taxonomic affiliations and metabolic potentials, shedding light on their suitability for various agricultural applications.The production of phytohormones, particularly indole acetic acid (IAA) and gibberellic acid (GA), by select microbial isolates highlights their potential as biofertilizers capable of enhancing plant growth and development. The ability of these microorganisms to solubilize phosphate further underscores their importance in improving soil fertility and nutrient availability to plants. The greenhouse experiments conducted as part of this study have demonstrated the practical utility of these microbial isolates in promoting plant growth, with significant improvements observed in shoot length, root length, biomass, and nutrient uptake in inoculated plants compared to controls. While the findings of this study are promising, further research is needed to fully realize the potential of microbial biofertilizers in agricultural systems. Field trials under diverse environmental conditions are necessary to validate the efficacy and stability of these microbial inoculants in real-world settings. Additionally, molecular studies aimed at elucidating the mechanisms underlying phytohormone production and phosphate solubilization can provide deeper insights into the functional roles of these microorganisms and inform strategies for their optimization. Environmental and ethical considerations must also be carefully addressed to ensure the safe and responsible use of microbial biofertilizers in agriculture. Regulatory frameworks and guidelines should be established to monitor and control their application, minimizing potential risks to ecosystems and human health. Public awareness and engagement efforts are crucial

for building trust and acceptance of these innovative agricultural technologies. The findings of this study contribute to the growing body of knowledge on plant-microbe interactions and offer promising solutions for enhancing agricultural sustainability and resilience. By harnessing the power of beneficial microorganisms, we can develop eco-friendly and cost-effective strategies to improve crop productivity, reduce dependency on chemical inputs, and promote soil health, ultimately contributing to global food security and environmental conservation efforts.

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