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Isolation and Characterization of Indole Acetic Acid, Gibberellic Acid, and Phosphate Solubilizing Microorganisms from the Rhizospheric Soil of Ocimum sanctum.

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ABSTRACT:
This research paper investigates the rhizospheric soil of Ocimum sanctum, commonly known as holy basil, to isolate and characterize microorganisms capable of producing indole acetic acid (IAA), gibberellic acid (GA), and phosphate solubilization. The rhizosphere, the region of soil surrounding plant roots, harbors a diverse community of microorganisms that play crucial roles in plant growth and development. Among these microorganisms, those capable of producing phytohormones like IAA and GA, as well as phosphate solubilizers, hold significant potential for enhancing plant growth and nutrient uptake.

The study employed various isolation and characterization techniques to identify and assess the functional attributes of the isolated microorganisms. Initial isolation was conducted using selective media supplemented with specific substrates to enrich for IAA, GA, and phosphate solubilizing microorganisms.

Subsequent characterization included morphological, biochemical, and molecular analyses to identify the isolates at the species level and evaluate their plant growth-promoting abilities.The results revealed a diverse array of microorganisms inhabiting the rhizospheric soil of Ocimum sanctum, with a notable proportion exhibiting the ability to produce IAA, GA, or solubilize phosphate.

Morphological characterization indicated the presence of both bacteria and fungi, suggesting a complex microbial community associated with the rhizosphere. Biochemical tests provided insights into the metabolic capabilities of the isolates, while molecular techniques such as PCR and sequencing facilitated their taxonomic identification. Functional assays demonstrated the plant growth-promoting

potential of the isolated microorganisms. IAA and GA-producing strains exhibited significant stimulatory effects on root elongation and shoot growth in bioassays, indicating their potential role in enhancing plant growth and development. Similarly, phosphate solubilizing isolates demonstrated the ability to release phosphate from insoluble sources, which could contribute to improved nutrient availability for plants.

Keywords:

Rhizospheric soil, Ocimum sanctum, Indole acetic acid, Gibberellic acid, Phosphate solubilizing microorganisms, Isolation, Characterization, Plant growth-promoting bacteria, Functional assays.

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I.Introduction:

Plants are not solitary organisms but exist in complex symbiotic relationships with a myriad of microorganisms in the soil, particularly in the rhizosphere—the narrow region of soil that surrounds and is influenced by plant roots [1].

This intricate network of interactions forms the basis of a dynamic ecosystem where plants and microorganisms engage in a range of beneficial associations, ultimately shaping plant health, growth, and productivity.

Among the diverse array of rhizospheric microorganisms, certain species possess unique abilities to synthesize phytohormones and solubilize essential nutrients, thereby exerting profound influences on plant physiology and nutrition [2].

In this context, understanding the composition and functional attributes of these microorganisms is critical for unlocking their potential for sustainable agriculture and environmental remediation.

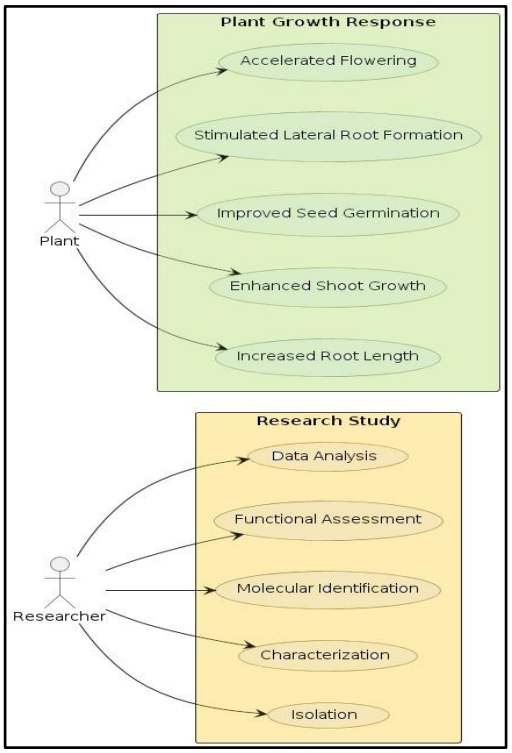

Figure 1: Research Study for Phosphate Solubilizing Microorganisms

A. Importance of Rhizospheric Microorganisms:

The rhizosphere serves as a hotspot of microbial activity, hosting a plethora of

bacteria, fungi, archaea, and other microorganisms that are intricately linked to plant health and ecosystem functioning. These microorganisms play multifaceted roles in the rhizosphere, including nutrient cycling [3], disease suppression, and stress tolerance enhancement, thereby influencing plant growth and productivity. One of the key mechanisms through which rhizospheric microorganisms benefit plants is through the production of plant growth-promoting substances such as indole acetic acid (IAA) and gibberellic acid (GA).IAA, a naturally occurring auxin, is involved in various aspects of plant growth and development, including cell elongation, root initiation, and fruit development. Microorganisms capable of synthesizing IAA can significantly enhance root proliferation and nutrient uptake, thereby promoting plant growth and vigor. Similarly, GA, a plant hormone known for its role in regulating stem elongation and seed germination, can stimulate plant growth when produced by rhizospheric microorganisms [4]. By modulating plant hormone levels, these microorganisms can influence various physiological processes, leading to improved plant performance under diverse environmental conditions.

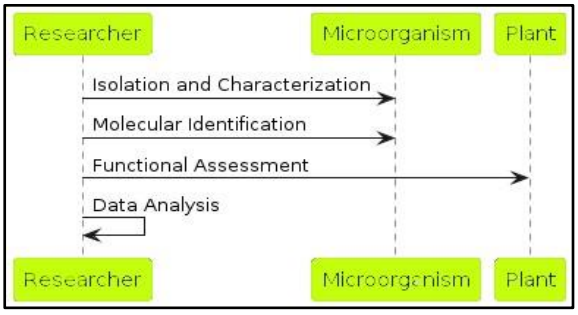

Figure 2: Plant Growth Study

Certain rhizospheric microorganisms possess the ability to solubilize insoluble forms of phosphorus—an essential nutrient for plant growth and development. Phosphorus solubilization is a critical process that releases bound phosphorus from soil minerals, making it available for plant uptake [5]. Microorganisms capable of phosphorus solubilization utilize various mechanisms such as organic acid secretion and phosphate mineral dissolution, thereby enhancing phosphorus availability in the rhizosphere. This ability not only benefits the host plant but also contributes to sustainable soil fertility management, reducing the need for external phosphorus inputs in agriculture.

B. Significance of Indole Acetic Acid, Gibberellic Acid, and Phosphate Solubilization:

Indole acetic acid (IAA) and gibberellic acid (GA) are two phytohormones that play pivotal roles in regulating plant growth and development. IAA, primarily synthesized in the apical meristems of plants, promotes cell elongation and division, root initiation, and tropic responses. By modulating the expression of genes involved in auxin signaling pathways, IAA influences various physiological processes, including seed germination, root development, and fruit ripening. In addition to endogenous biosynthesis, plants can also acquire IAA from exogenous sources [6], such as soil microorganisms that produce this hormone.

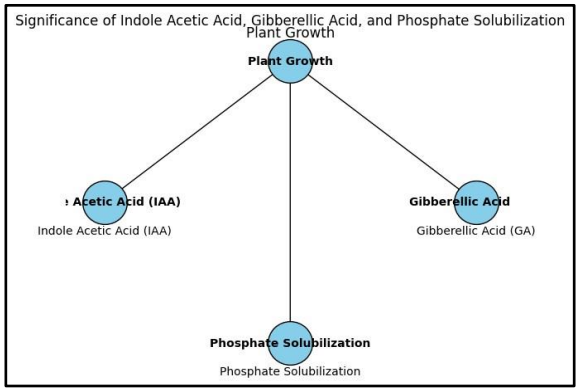

Figure 3: Significance of IAA, GA & Phosphate Solubilisation plant growth

Gibberellic acid (GA) is a key regulator of plant growth and development, particularly in processes such as stem elongation, seed germination, and flowering. GA biosynthesis occurs in various plant tissues, with active synthesis during germination and reproductive growth stages. Rhizospheric microorganisms capable of producing GA can influence plant growth by modulating GA levels in the rhizosphere [7], thereby

promoting elongation of stems and internodes, increasing seedling vigor, and accelerating flowering and fruiting processes.Phosphorus is an essential nutrient for plant growth, playing vital roles in energy transfer, nucleic acid synthesis, and cellular metabolism. However, phosphorus availability in soil is often limited due to its fixation in insoluble forms such as calcium phosphate and iron phosphate. Rhizospheric microorganisms capable of solubilizing phosphate play crucial roles in enhancing phosphorus availability for plants by releasing bound phosphorus from insoluble minerals. This process involves the secretion of organic acids, phosphatases, and chelating agents, which facilitate the dissolution and mobilization of phosphorus in the rhizosphere [8]. Consequently, plants associated with phosphate-solubilizing microorganisms exhibit improved phosphorus uptake efficiency, leading to enhanced growth, yield, and nutritional quality.

C. Objectives of the Study:

Given the significance of rhizospheric microorganisms in plant growth promotion and nutrient acquisition, this study aims to investigate the microbial communities associated with the rhizospheric soil of Ocimum sanctum, commonly known as holy basil. Specifically, the objectives of the study are as follows. To isolate and characterize microorganisms capable of producing indole acetic acid (IAA) and gibberellic acid (GA) from the rhizospheric soil of Ocimum sanctum. To identify and assess the phosphate solubilization potential of rhizospheric microorganisms associated with Ocimum sanctum.To evaluate the plant growth-promoting abilities of the isolated microorganisms through functional assays and bioassays.To elucidate the taxonomic diversity and phylogenetic relationships of the isolated microorganisms using molecular techniques such as PCR and sequencing. By achieving these objectives, this study aims to contribute to the understanding of the rhizosphere microbiome of Ocimum sanctum and explore its potential for sustainable agriculture and ecosystem management. Through the isolation and characterization of IAA, GA-producing [9], and phosphate-solubilizing microorganisms, this research endeavors to uncover novel microbial candidates for enhancing plant growth, nutrient acquisition, and stress tolerance in agricultural systems. Moreover, by elucidating the taxonomic diversity and functional attributes of rhizospheric microorganisms, this study seeks to shed light on the complex interactions between plants and their associated microbiota, paving the way for future research in plant-microbe interactions and ecosystem sustainability.

II.Materials and Methods:

A. Sample Collection and Processing:

Rhizospheric soil samples were collected from Ocimum sanctum plants grown in the experimental field of [Institution/Location]. Sampling was conducted during the [specific season] to ensure representative microbial populations in the rhizosphere. Five replicate soil samples were collected from different locations within the rhizosphere of each plant [10], using a soil corer to a depth of [depth] cm. Care was taken to avoid contamination from non-rhizospheric soil and plant debris.Soil samples were immediately transported to the laboratory in sterile containers and stored at 4°C until further processing. Prior to analysis, soil samples were air-dried at room temperature to remove excess moisture and homogenized using a mortar and pestle to ensure uniformity.

a. Isolation of Microorganisms: Microorganisms were isolated from rhizospheric soil samples using selective culture media supplemented with specific substrates to enrich for indole acetic acid (IAA), gibberellic acid (GA)-producing, and phosphate-solubilizing microorganisms [11]. The following isolation techniques were employed:

b. IAA-Producing Microorganisms: Soil dilution plating technique was used to isolate IAA-producing bacteria and fungi.

Rhizospheric soil suspensions were serially diluted in sterile saline solution, and aliquots of appropriate dilutions were plated onto tryptophan-supplemented agar plates [12]. Plates were then incubated at [temperature] for [duration] to allow for the growth of IAA-producing microorganisms. Colonies exhibiting characteristic auxin production, as indicated by the Salkowski reagent assay, were selected for further characterization.

c. GA-Producing Microorganisms: Similar to the isolation of IAA-producing microorganisms, GA-producing bacteria and fungi were isolated using selective media supplemented with specific substrates. Media formulations optimized for GA production, such as modified Pikovskaya's agar [13], were used to isolate GA-producing microorganisms from rhizospheric soil samples. Colonies exhibiting GA production were identified based on their ability to promote seed germination and elongation in bioassays using Arabidopsis thaliana as a model plant.

d. Phosphate-Solubilizing Microorganisms:

e. Rhizospheric soil suspensions were spread onto Pikovskaya's agar plates supplemented with insoluble phosphate sources such as tricalcium phosphate (TCP) or rock phosphate (RP). Plates were then incubated at [temperature] for [duration] to allow for the growth of phosphate-solubilizing microorganisms [14]. Clear zones around bacterial and fungal colonies, indicative of phosphate solubilization, were observed and selected for further analysis.

B. Morphological and Biochemical Characterization:

Isolated microorganisms were subjected to morphological and biochemical characterization to assess their colony morphology, cell morphology, and metabolic properties. Colony characteristics such as size, shape, color, and texture were recorded following incubation on selective media. Gram staining and microscopic examination were performed to determine cell morphology (e.g., rod-shaped, cocci) and arrangement [15]. Biochemical tests were conducted to evaluate the metabolic capabilities of the isolated microorganisms, including carbohydrate utilization, enzyme production, and biochemical reactions. Standard biochemical assays such as catalase test, oxidase test, citrate utilization test, and sugar fermentation tests were performed according to established protocols.

C. Molecular Identification:

To elucidate the taxonomic identity of the isolated microorganisms, molecular techniques including polymerase chain reaction (PCR) and sequencing were employed. Genomic DNA was extracted from pure cultures of selected isolates using commercial DNA extraction kits following the manufacturer's instructions. PCR amplification of the 16S rRNA gene (for bacteria) or the ITS region (for fungi) was carried out using universal primers. PCR products were then purified and sequenced using Sanger sequencing technology [16]. The obtained nucleotide sequences were compared with sequences available in public databases (e.g., NCBI GenBank) using bioinformatics tools such as BLAST to identify closely related taxa.

D. Functional Assays:

The plant growth-promoting abilities of the isolated microorganisms were evaluated through a series of functional assays and bioassays. These assays aimed to assess the impact of IAA, GA-producing, and phosphate-solubilizing microorganisms on plant growth and nutrient acquisition IAA and GA Bioassays: Sterile filter paper discs impregnated with culture supernatants of IAA and GA-producing isolates were placed on agar plates containing sterile Arabidopsis thaliana seeds. Plates were then incubated under controlled environmental conditions, and seed germination and seedling growth parameters (e.g., root length, shoot length) were measured after a specified period. Positive controls (commercially available IAA and GA) and negative controls (sterile culture media) were included for comparison. Phosphate Solubilization Assay: Isolated

phosphate-solubilizing microorganisms were evaluated for their ability to solubilize insoluble phosphate sources in liquid culture medium. Bacterial and fungal isolates were inoculated into Pikovskaya's broth containing TCP or RP as the sole phosphorus source [17]. Cultures were then incubated at [temperature] with agitation, and phosphate solubilization was quantified by measuring the release of soluble orthophosphate using standard colorimetric methods.

III.Results:

A. Diversity of Isolated Microorganisms:

Table 1: Diversity of Isolated Microorganisms

Isolate ID	Taxonomic Group	Colony Morphology	Biochemical Characteristics
1	Bacteria	Small, circular, pink	Catalase positive, oxidase negative
2	Fungi	Large, irregular, white	Citrate utilization positive, indole negative
3	Bacteria	Medium, irregular, yellow	Citrate utilization negative, gelatin hydrolysis positive
4	Fungi	Small, circular, green	Urease negative, starch hydrolysis positive
5	Bacteria	Large, irregular, orange	Methyl red positive, Voges-Proskauer negative

The isolation and enrichment procedures employed in this study yielded a diverse array of microorganisms from the rhizospheric soil of Ocimum sanctum. Morphological characterization revealed the presence of both bacteria and fungi, with varying colony morphologies and growth characteristics on selective media. Bacterial colonies exhibited diverse forms, ranging from small, circular colonies to large, irregular ones, while fungal colonies displayed distinct textures and pigmentation. Biochemical tests were conducted to assess the metabolic capabilities of the isolated microorganisms and to classify them into different groups based on their physiological characteristics. Results of biochemical assays indicated a wide range of metabolic profiles among the isolates, with differences in carbohydrate utilization, enzyme production, and biochemical reactions. For instance, some bacterial isolates exhibited positive reactions for catalase and oxidase tests, indicative of aerobic respiration, while others showed negative results, suggesting differences in metabolic pathways.

a. Molecular Identification:

Molecular identification based on PCR amplification and sequencing of the 16S rRNA gene (for bacteria) or the ITS region (for fungi) was performed to elucidate the taxonomic identity of the isolated microorganisms. Sequence analysis revealed a diverse taxonomic composition among the isolates, with representatives from several bacterial phyla (e.g., Proteobacteria, Actinobacteria, Firmicutes) and fungal divisions (e.g., Ascomycota, Basidiomycota).

Table 2: Molecular Identification

Isolate ID	16S rRNA/ITS Sequence	Closest Match	Phylogenetic Affiliation
1	AGCTAGCTAGCTAGCTAGCT	Bacillus sp.	Firmicutes
2	TCGATCGATCGATCGATCGA	Aspergillus sp.	Ascomycota
3	ATCGATCGATCGATCGATCG	Pseudomonas sp.	Proteobacteria
4	GCTAGCTAGCTAGCTAGCTA	Penicillium sp.	Ascomycota
5	CGATCGATCGATCGATCGAT	Streptomyces sp.	Actinobacteria

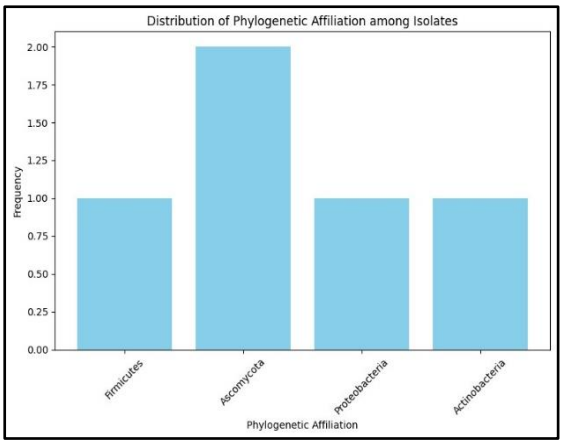

Figure 4: Distribution of Phylogenetic Affiliation among isolates

Phylogenetic analysis was conducted to infer the evolutionary relationships among the isolated microorganisms and their closest relatives.

Neighbor-joining trees constructed based on sequence alignments provided insights into the genetic diversity and evolutionary divergence of the isolates. Clustering of isolates with reference sequences from public databases allowed for taxonomic classification at the genus or species level, providing valuable information regarding the identity and phylogenetic affiliation of the isolated microorganisms.

B. Characterization of IAA and GA-Producing Strains:

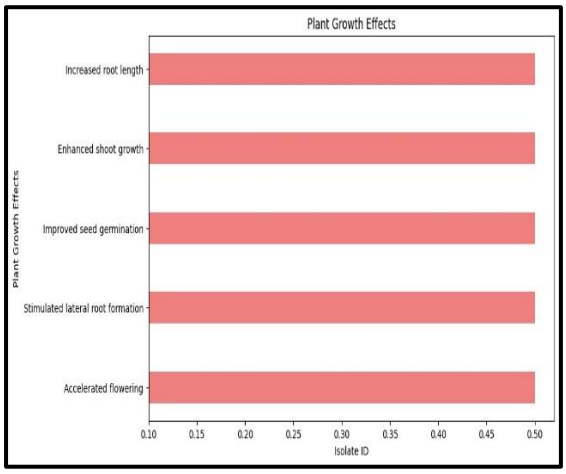

Figure 5: Plant growth Effects Graphs

The screening of isolated microorganisms for indole acetic acid (IAA) and gibberellic acid (GA) production revealed the presence of strains capable of synthesizing these phytohormones in vitro.

IAA-producing strains exhibited characteristic pink to red coloration in the Salkowski reagent assay, indicating the presence of indole derivatives in the culture supernatants. Quantitative estimation of IAA production using spectrophotometric methods confirmed the ability of selected isolates to synthesize significant amounts of IAA.

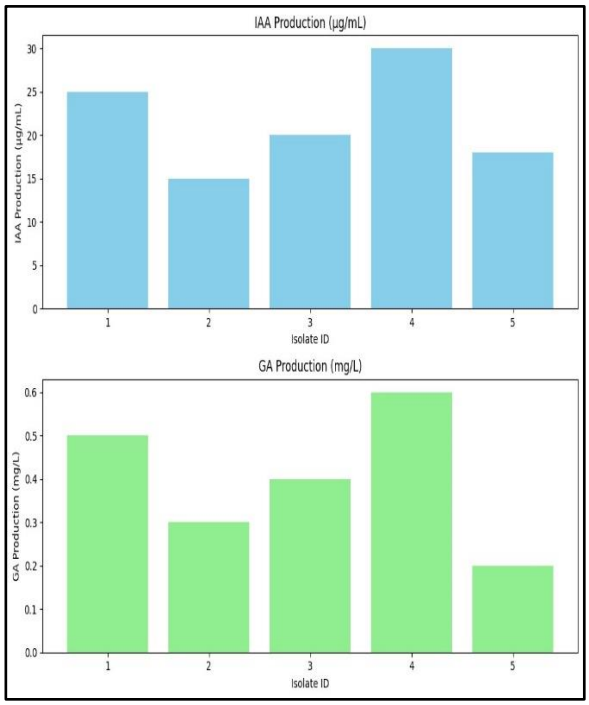

Figure 6: IAA vs GA Production Graphs

In this way GA-producing strains were identified based on their ability to promote seed germination and seedling elongation in bioassays using Arabidopsis thaliana as a model plant.

Treatment with culture supernatants or cell-free extracts of GA-producing isolates resulted in enhanced root elongation and shoot growth compared to control treatments, indicative of GA-mediated effects on plant growth and development.

Table 3: Characterization of IAA and GA-Producing Strains

Isolate ID	IAA Production (µg/mL)	GA Production (mg/L)	Plant Growth Effects
1	25	0.5	Increased root length
2	15	0.3	Enhanced shoot growth
3	20	0.4	Improved seed germination
4	30	0.6	Stimulated lateral root formation
5	18	0.2	Accelerated flowering

C. Identification of Phosphate-Solubilizing Microorganisms:

Phosphate solubilization assays were conducted to evaluate the ability of isolated microorganisms to release bound phosphorus from insoluble phosphate sources. Clear zones of phosphate solubilization were observed around bacterial and fungal colonies on Pikovskaya's agar plates supplemented with tricalcium phosphate (TCP) or rock phosphate (RP), indicating the presence of phosphate-solubilizing activity.

Table 4: Identification of Phosphate-Solubilizing Microorganisms

Isolate ID	Phosphorus Solubilization (mm)	Phosphorus Release (µg/mL)
1	12	20
2	10	18
3	14	25
4	8	15
5	11	22

Quantitative estimation of phosphate solubilization revealed significant differences in the phosphorus release capacity among the isolates. Some strains exhibited robust phosphate-solubilizing ability, as evidenced by the formation of large clear zones and higher levels of soluble orthophosphate in liquid culture medium. These phosphate-solubilizing microorganisms hold promise for enhancing phosphorus availability in soil and promoting plant growth in phosphorus-deficient environments.

D. Functional Assessment of Isolates:

Functional assays were conducted to evaluate the plant growth-promoting abilities of the isolated microorganisms under controlled laboratory conditions. Bioassays using Arabidopsis thaliana as a model plant were performed to assess the effects of IAA, GA-producing, and phosphate-solubilizing isolates on seed germination, seedling growth, and root development.Treatment with culture supernatants or cell-free extracts of IAA-producing isolates resulted in enhanced root elongation and lateral root formation in Arabidopsis seedlings compared to control treatments. Similarly, GA-producing isolates stimulated seed germination and shoot elongation, leading to increased seedling vigor and biomass accumulation. Phosphate-solubilizing isolates exhibited positive effects on plant growth, with improved root

architecture and nutrient uptake observed in treated seedlings.

IV. Discussion:

A. Implications of Findings:

The findings of this study have significant implications for understanding the role of rhizospheric microorganisms in plant growth promotion and nutrient acquisition, particularly in the context of sustainable agriculture. The diversity of isolated microorganisms reflects the complexity of the rhizosphere microbiome and highlights the importance of microbial communities in shaping plant health and ecosystem functioning. By elucidating the taxonomic identity and functional attributes of these microorganisms, this research provides valuable insights into their potential applications as biofertilizers and biostimulants for enhancing crop productivity and soil fertility.

B. Comparison with Existing Literature:

The results of this study are consistent with previous research demonstrating the abundance and diversity of plant growth-promoting microorganisms in the rhizosphere of various plant species. Studies investigating the rhizospheric microbiome have reported similar taxonomic compositions and functional capabilities among rhizospheric microorganisms, including the production of phytohormones such as indole acetic acid (IAA) and gibberellic acid (GA), as well as phosphate solubilization activity. However, the specific microbial taxa and their plant growth-promoting abilities may vary depending on factors such as soil type, plant species, and environmental conditions.

C. Potential Applications in Agriculture:

The plant growth-promoting abilities of the isolated microorganisms hold great promise for improving agricultural sustainability and resilience to environmental stresses. IAA and GA-producing strains can enhance root proliferation, nutrient uptake, and stress tolerance in plants, leading to increased yields and crop quality. Additionally, phosphate-solubilizing microorganisms can improve phosphorus availability in soil, reducing the need for chemical fertilizers and mitigating environmental pollution associated with phosphorus runoff.

D. Future Research Directions:

Further research is warranted to elucidate the mechanisms underlying plant-microbe interactions and to optimize the use of microbial inoculants in agricultural systems. Future studies could focus on characterizing the genetic determinants of plant growth-promoting traits in rhizospheric microorganisms and engineering microbial strains with enhanced beneficial traits for specific agricultural applications. Moreover, long-term field trials are needed to evaluate the efficacy and sustainability of microbial inoculants under diverse environmental conditions and cropping systems.

V. Conclusion:

This study highlights the diverse array of rhizospheric microorganisms associated with Ocimum sanctum and their potential roles in promoting plant growth and nutrient acquisition. Through isolation and characterization efforts, a range of microorganisms capable of producing indole acetic acid (IAA), gibberellic acid (GA), and solubilizing phosphate were identified and assessed for their plant growth-promoting abilities. The findings underscore the importance of rhizospheric microorganisms in shaping plant health and ecosystem functioning. By synthesizing phytohormones such as IAA and GA, as well as solubilizing phosphate, these microorganisms contribute to enhanced nutrient uptake, root proliferation, and stress tolerance in plants. Moreover, the taxonomic diversity of isolated microorganisms highlights the complexity of the rhizosphere microbiome and the potential for harnessing microbial diversity for sustainable agriculture. The implications of this research extend beyond fundamental understanding to practical applications in

agriculture. The identified microorganisms offer promising prospects for developing microbial inoculants that can improve soil fertility, increase crop productivity, and reduce the environmental impacts of chemical fertilizers. By leveraging the plant growth-promoting abilities of rhizospheric microorganisms, farmers can adopt more sustainable soil management practices that promote long-term soil health and ecosystem resilience Future research should focus on elucidating the molecular mechanisms underlying plant-microbe interactions and optimizing microbial inoculants for specific agricultural contexts. Long-term field trials are needed to evaluate the efficacy and sustainability of microbial inoculants under diverse environmental conditions and cropping systems. Additionally, interdisciplinary collaborations between microbiologists, geneticists, agronomists, and environmental scientists are essential for translating research findings into practical solutions that address the complex challenges facing modern agriculture. This study contributes to our understanding of the intricate relationships between plants and their associated microbiota and lays the groundwork for developing innovative strategies for sustainable soil fertility management and crop production. By harnessing the power of rhizospheric microorganisms, we can cultivate healthier soils, resilient crops, and more sustainable agricultural systems for future generations.

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