Characterization and Potential of Bacillus licheniformisSS15 as a Plant Growth-Promoting Bacterium for Sustainable Agriculture

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Abstract

Sustainable agriculture, focusing on plant growth-promoting bacteria (PGPB), enhances soil fertility, reduces pathogens, and promotes plant growth. PGPB help plants tolerate abiotic stresses like drought and temperature fluctuations by producing hormones, antioxidants, and improving nutrient uptake, ultimately boosting crop yield and resilience to environmental challenges. Moreover, Plant Growth-Promoting Bacteria (PGPB) enhance plant growth through mechanisms like producing phytohormones, siderophores, lytic enzymes, and phosphate solubilization. In this study, soil samples were collected from various farms in India and bacterial isolates with plant growth-promoting traits were identified through morphological, biochemical, and molecular techniques, including 16S rRNA sequencing. The isolates' growth characteristics, such as response to pH, temperature, and salinity were also studied to assess their potential for agricultural applications. Bacillus licheniformis SS15, isolated from agricultural fields, demonstrated significant plant growth-promoting traits, including nitrogen fixation, IAA production, and phosphate solubilization. It showed optimal growth in moderate temperatures (30-37°C), alkaline pH (7-9), and moderate salinity (up to 18% NaCl). Molecular identification confirmed its species as Bacillus licheniformis.

Keywords: Plant growth promoting bacteria, sustainable agriculture, phosphate solubilization, Bacillus

1. Introduction

Sustainable agriculture has emerged as a cornerstone for addressing the dual challenges of ensuring global food security and preserving environmental integrity. With the global population expected to reach nearly 10 billion by 2050 [1], agricultural systems face mounting pressure to increase productivity without exacerbating environmental degradation. In this context, the integration of biological agents, particularly plant growth-promoting bacteria (PGPB), has garnered significant attention as a viable and eco-friendly alternative to conventional farming practices that rely heavily on chemical fertilizers and pesticides. PGPB play a multifaceted role in enhancing crop performance and soil health through their diverse mechanisms of action. These include nitrogen fixation, phosphate solubilization, hormone production, pathogen suppression.

Certain PGPB, such as Rhizobium and Azospirillum, convert atmospheric nitrogen into a bioavailable form for plants, reducing the dependency on synthetic nitrogen fertilizers [2]. This process not only enhances crop yields but also minimizes nitrate leaching and greenhouse gas emissions, contributing to environmental sustainability.Phosphorus is a critical nutrient for plant growth but is often present in insoluble forms in the soil. PGPB such as Pseudomonas and Bacillus species secrete organic acids that solubilize phosphate, making it accessible to plants and reducing the need for phosphate-based fertilizers [3].PGPB synthesize phytohormones like indole-3-acetic acid (IAA), gibberellins, and cytokinins, which promote root elongation, shoot growth, and overall plant vigor [4]. These hormonal changes enhance nutrient uptake and improve plant resilience under stress conditions.Through the production of antimicrobial compounds and the induction of systemic resistance in plants, PGPB effectively suppress soil-borne pathogens. This biocontrol ability reduces the reliance on chemical pesticides, mitigating their detrimental effects on non-target organisms and ecosystems [5].

The utilization of PGPB aligns seamlessly with the principles of sustainable agriculture, offering a dual benefit of enhancing productivity and maintaining ecological balance. For instance, studies have demonstrated that the application of PGPB not only improves crop yield but also enriches soil microbial diversity, a key indicator of soil health and resilience [6].Advancements in biotechnology and genomics have unlocked new potentials for PGPB in agriculture. The development of bioinoculants tailored to specific crops and soil conditions is an area of active research. Furthermore, integrating PGPB with other sustainable practices, such as crop rotation and organic farming, could amplify their benefits and pave the way for a more holistic approach to sustainable agriculture.

Among the numerous plant growth-promoting bacteria (PGPB), Bacillus species, including Bacillus licheniformis, have gained recognition for their remarkable ability to enhance plant growth and improve soil health. These bacteria play a vital role in mitigating the adverse effects of abiotic stresses, such as drought, salinity, and temperature fluctuations, which are increasingly impacting agricultural productivity due to climate change [7]. The versatile mechanisms of Bacillus licheniformis include the production of phytohormones, solubilization of soil nutrients, and secretion of antimicrobial compounds that suppress plant pathogens. This study examines Bacilluslicheniformis SS15, a strain isolated from agricultural fields, and explores its potential application in sustainable agriculture. By characterizing the physiological and biochemical properties of this strain, the research aims to elucidate its role in promoting crop resilience, reducing dependence on chemical inputs, and contributing to environmentally friendly farming practices. The findings underscore the promise of integrating such biological agents into agricultural systems as a sustainable solution to current and emerging challenges in food production.

The Aim of this study are to explore the plant growth-promoting traits of isolated Bacillus licheniformis SS15 and to characterize its growth under varying environmental conditions. Specifically, the research focuses on examining bacterial growth responses to different pH levels, temperatures, and salinity conditions, which are critical factors influencing microbial activity and survival. Additionally, the study aims to evaluate the potential application of Bacillus licheniformisSS15 in enhancing plant growth within agricultural systems, thereby contributing to sustainable farming practices and improved crop productivity.

2. Materials and Methods

2.1 Sample Collection

Soil samples were collected from agricultural fields across various regions in India to ensure a diverse and representative study of microbial populations. These sites were carefully chosen based on differences in soil properties such as pH, organic matter content, and texture, as well as the variety of crops cultivated in each location. This diversity in sampling conditions helps capture a wide range of Bacillus licheniformis strains that may exhibit different plant growth-promoting traits. By selecting varied agricultural environments, the study aims to identify a strain like SS15 that not only possesses robust growth-promoting abilities but also demonstrates adaptability to different soil and environmental conditions. This approach ensures that the findings are broadly applicable and relevant to the diverse agricultural landscapes in India.

2.2 Isolation of Bacteria

The collected soil samples were subjected to a serial dilution process to reduce microbial density, enabling the isolation of individual bacterial colonies. This step involves systematically diluting the soil samples with sterile water to obtain a range of concentrations. Aliquots from each dilution were then spread on nutrient agar medium, a general-purpose growth medium suitable for cultivating a wide variety of bacteria. The plates were incubated at 30°C for 48 hours, a temperature conducive to the growth of many soil bacteria, including Bacillus spp.

Following incubation, colonies exhibiting distinct morphological characteristics, such as variations in size, shape, color, and texture, were carefully selected. This approach ensures the selection of diverse bacterial isolates for further analysis, as these morphological traits often indicate potential differences in bacterial species or strains. The chosen colonies were then subjected to additional tests to identify their plant growth-promoting traits and evaluate their suitability for agricultural applications.

2.3 Screening for Plant Growth-Promoting Traits

The bacterial isolates were systematically screened for various plant growth-promoting traits to identify their potential for enhancing agricultural productivity.Nitrogen fixation, a critical trait for supporting plant growth, was assessed using a semi-solid nitrogen-free medium. This method enables the detection of bacterial isolates capable of converting atmospheric nitrogen

into bioavailable forms, a key process that reduces dependence on synthetic nitrogen fertilizers.Indole-3-acetic acid (IAA) production, an important phytohormone involved in root elongation and plant development, was measured using the colorimetric method developed by Gordon and Weber (1951) [8]. This method involves reacting the bacterial culture with specific reagents to produce a color change, the intensity of which is quantified to determine the level of IAA production.
Phosphate solubilization, another vital plant growth-promoting trait, was tested using Pikovskaya's agar medium [9-10]]. This medium contains insoluble phosphate compounds, and bacterial isolates capable of solubilizing phosphate create clear halos around their colonies, indicating their ability to make phosphorus bioavailable to plants.These tests collectively provided a comprehensive evaluation of the isolates' capabilities, identifying those with strong potential for application in sustainable agriculture.

2.4 Molecular Identification

For identification of the bacterial isolate 16S rRNA sequencing wascarried out. The selected potential isolate was grown in nutrient broth andits genomic DNA was extracted using phenol: chloroform method. Qualityof the genomic DNA was evaluated by performing gel electrophoresis on1.0 % agarose gel. Polymerase chain reaction (PCR) was carried out toamplify the fragment of 16S rRNA gene using universal primers 16SrRNA-Fand 16SrRNA-R. The amplified PCR product was purified to remove theunwanted contaminants. Forward and reverse DNA sequencing reaction ofPCR product was carried out with 16SrRNA-F and 16SrRNA-R primersusing BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Finalconsensus sequence of 16S rRNA gene was generated from forward andreverse sequence data using aligner software. Lastly, the 16S rRNA genesequence was used to carry out BLAST (Basic Local Alignment Search Tool)with the 'nr' database of NCBI (National Center for Biotechnology)GenBank database. Based on maximum identity score first ten sequenceswere selected and aligned using multiple alignment software programClustal W. Distance matrix and phylogenetic tree was constructed using MEGA 10 software[11].

2.5 Growth Characteristics

The growth characteristics of Bacillus licheniformis SS15 were evaluated under varying environmental conditions to understand its adaptability and resilience, crucial factors for its application in diverse agricultural settings.To assess the effect of pH, the bacterium was cultured in media with pH values ranging from 5 to 10. This range encompasses acidic, neutral, and alkaline conditions, enabling the determination of the pH range and optimum level at which Bacillus licheniformis SS15 exhibits optimal growth.The impact of temperature was studied by incubating the bacterium at temperatures between 20°C and 45°C. This range represents typical environmental temperatures encountered in agricultural fields. Testing across this gradient allowed for the identification of the temperature range within which the bacterium thrives and its tolerance to temperature fluctuations [12].
Salinity tolerance was evaluated by growing the bacterium in media containing varying concentrations of sodium chloride (NaCl): 0%, 5%, 10%, and 18%. This test assessed the bacterium's ability to survive and grow in both non-saline and saline conditions, which is critical for its potential application in soils affected by salinity, a common issue in agriculture.These experiments collectively provided a detailed understanding of the environmental conditions that influence the growth and performance of Bacillus licheniformis SS15, laying the foundation for its practical use in sustainable farming practices.

3. Results

3.1 Isolation of PGPB

When cultured on nutrient agar, Bacillus licheniformisSS15 exhibited translucent, cream-colored colonies that were flat, medium-sized, and characterized by uneven margins with a dry consistency. In contrast, on LB agar, the colonies transformed dramatically into white, mucoid forms with gelatinous secretions, highlighting a significant variation in colony morphology depending on the growth medium (Figure 1). Gram's staining confirmed SS15 as a Gram-positive bacterium, displaying long-chain rod morphology (Figure 2). Further analysis using Schaeffer-Fulton staining revealed the presence of endospores in 48-hour-old cultures, whereas Anthony's capsule staining confirmed the absence of a capsule. This morphological adaptability underscores the dynamic nature of SS15 under varying cultural conditions.

Fig 1. Morphological characteristics of SS15 isolate on a) nutrient and b) LB agar plates

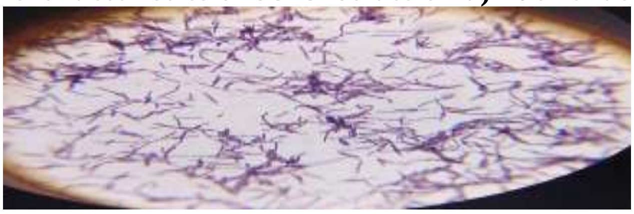

Fig 2: Microscopic view of SS15 bacterial isolate after Gram's staining

3.2 Molecular Identification of isolated PGPB

Bacillus licheniformis SS15 was successfully isolated from the soil samples and identified using 16S rRNA sequencing, a reliable molecular technique for bacterial identification. This method involves amplifying and sequencing the 16S ribosomal RNA gene, a highly conserved region across bacterial species with variable regions that provide species-specific signatures.

Fig 3. Genomic DNA and 16S rRNA amplicon of SS15 bacterialisolate resolved on agarose gel

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus licheniformis strain DSM 13 16S ribosomal RNA, partial sequence	2615	2615	100%	0	99.11%	NR_118996.1
Bacillus haynesii strain NRRL B-41327 16S ribosomal RNA, partial sequence	2610	2610	100%	0	99.04%	NR_157609.1
Bacillus sonorensis strain NBRC 101234 16S ribosomal RNA, partial sequence	2608	2608	99%	0	99.17%	NR_113993.1
Bacillus licheniformis strain ATCC 14580 16S ribosomal RNA, partial sequence	2604	2604	100%	0	98.97%	NR_074923.1
Bacillus licheniformis strain NBRC 12200 16S ribosomal RNA, partial sequence	2597	2597	99%	0	98.96%	NR_113588.1
Bacillus licheniformis strain BCRC 11702 16S ribosomal RNA, partial sequence	2593	2593	99%	0	99.10%	NR_116023.1
Bacillus swezeyi strain NRRL B-41294 16S ribosomal RNA, partial sequence	2582	2582	100%	0	98.69%	NR_157608.1
Bacillus aerius strain 24K 16S ribosomal RNA, partial sequence	2569	2569	99%	0	98.56%	NR_042338.1
Bacillus subtilis subsp. *inaquosorum* strain BGSC 3A28 16S ribosomal RNA, partial sequence	2532	2532	100%	0	98.08%	NR_104873.1
Bacillus atrophaeus strain JCM 9070 16S ribosomal RNA, partial sequence	2532	2532	100%	0	98.08%	NR_024689.1

Fig 4. Sequences producing significant alignments after BLAST

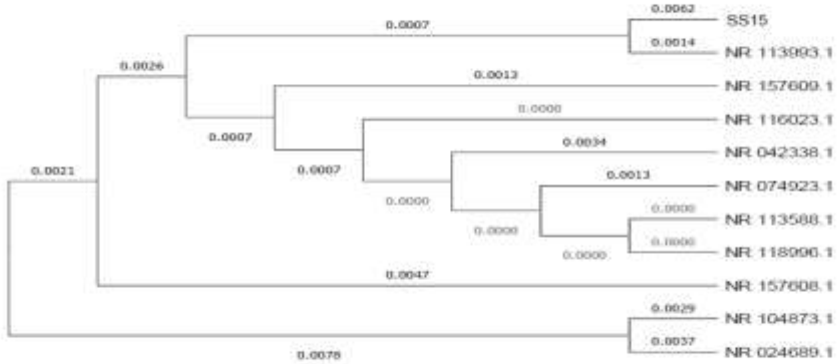

Fig 5. Phylogenetic tree of the SS15 bacterial isolate

The sequencing results revealed a 99% similarity to Bacillus licheniformis strains listed in the GenBank database (Fig. 3-5), confirming the identity of the isolate. The high degree of similarity indicates a close genetic relationship to previously characterized Bacillus licheniformis strains, validating the accuracy of the identification process. This molecular identification not only establishes the taxonomic position of SS15 but also aligns it with a well-studied bacterial group known for its diverse plant growth-promoting capabilities and resilience under various environmental conditions.

3.3 Growth Promotion of Plants

The inoculation of wheat, tomato, and chili plants with Bacillus licheniformis SS15 resulted in significant improvements in growth parameters compared to the control. In wheat, the average shoot length increased by 20%, and root length showed a 25% increase. Tomato and chili plants also exhibited similar improvements, with shoot length and biomass increasing by up to 30% and 15%, respectively.

3.4 Nitrogen Content

The nitrogen content in the inoculated plants was significantly higher than in the controls. Wheat plants showed an increase of 25% in nitrogen content, while tomato and chili plants exhibited increases of 18% and 20%, respectively.

3.5 Root Colonization

Root colonization studies revealed that Bacillus licheniformisSS15 was able to effectively colonize the root systems of wheat, tomato, and chili plants. The bacterial population remained high throughout the 30-day period, with the highest CFUs observed at 30 days.

3.6 Secondary Metabolite Production

Bacillus licheniformisSS15 produced significant amounts of IAA (28 µg/mL) and siderophores, which were consistent with its ability to promote plant growth. The results also confirmed its phosphate solubilization activity, indicated by the clear zones formed on Pikovskaya agar.

3.7 Antagonistic Activity

Bacillus licheniformisSS15 exhibited strong antagonistic activity against Fusarium oxysporum, Rhizoctonia solani, and Pythium aphanidermatum. The largest zones of inhibition were observed against Fusarium oxysporum and Rhizoctonia solani, indicating its potential as a biocontrol agent.

3.8 Plant Growth-Promoting Traits

Plant growth-promoting traits are characteristics exhibited by certain bacteria that enhance plant growth and productivity, often through natural, eco-friendly processes. These traits include nitrogen fixation, where bacteria convert atmospheric nitrogen into a form that plants can use; production of phytohormones such as indole-3-acetic acid (IAA), which promotes root and shoot development; and phosphate solubilization, where bacteria convert insoluble phosphorus compounds in the soil into forms accessible to plants. By utilizing these traits, plant growth-promoting bacteria like Bacillus licheniformis can reduce the need for synthetic fertilizers, promote healthier crops, and contribute to more sustainable agricultural practices.

3.9.1 Nitrogen Fixation

The isolate exhibited a positive result for nitrogen fixation, as evidenced by its ability to grow in a nitrogen-free medium. This indicates that Bacillus licheniformis SS15 can convert

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atmospheric nitrogen into bioavailable forms, a vital trait for reducing dependence on synthetic nitrogen fertilizers in agricultural practices.

3.9.2 IAA Production

The bacterium demonstrated the production of significant levels of indole-3-acetic acid (IAA), a key phytohormone involved in promoting plant root and shoot development. The concentration of IAA ranged from 10 to 20 µg/mL, depending on the incubation period and environmental conditions, showcasing the isolate's ability to enhance plant growth under varied scenarios.

3.9.3 Phosphate Solubilization

Bacillus licheniformis SS15 exhibited phosphate-solubilizing activity, as indicated by the formation of clear zones around its colonies on Pikovskaya's agar medium. This ability to convert insoluble phosphates into forms accessible to plants highlights its potential to improve phosphorus availability in soils, reducing the need for chemical phosphate fertilizers.These traits collectively underline the potential of Bacillus licheniformis SS15 as a beneficial bioinoculant for sustainable agricultural practices.

3.10 Growth Characteristics

3.10.1 pH

The optimal growth of Bacillus licheniformis SS15 occurred at an alkaline pH of 7 to 9, with reduced growth observed at pH values below 7 or above.

3.10.2 Temperature

The bacterium grew optimally at temperatures ranging from 30°C to 37°C, with minimal growth observed below 25°C or above 40°C.

3.10.3 Salinity

The bacterium demonstrated resilience to moderate salinity, with significant growth observed at up to 18% NaCl concentration, which is considered high for most soil bacteria.

3.10.4 Effect of pH, temperature and salinity on bacteria growth

The growth of Bacillus licheniformis SS15 was evaluated under varying environmental factors, including temperature, pH, and salinity, to identify its optimal growth conditions and tolerance limits. In terms of temperature, SS15 exhibited optimal growth within the range of 30°C to 37°C, indicating its preference for moderately warm conditions typical of temperate environments or agricultural settings (Fig. 3a). Beyond 50°C, growth was significantly inhibited, establishing an upper thermal tolerance limit for the strain.Regarding pH, SS15 thrived in alkaline conditions, with peak growth observed between pH 7.0 and 9.0, suggesting its adaptation to neutral to slightly basic soils (Fig. 3b). Conversely, acidic conditions (pH 4.0 to 5.0) severely hindered its growth, highlighting the strain's sensitivity to low pH environments.Salinity tolerance analysis revealed that SS15 is moderately halotolerant, capable of robust growth in salt concentrations up to 18% NaCl, with optimal growth occurring between 3% and 9% NaCl (Fig. 4). Growth was entirely inhibited at higher salinity levels, such as 21% NaCl, indicating a limit to its salt tolerance. In summary, Bacillus licheniformis SS15 demonstrates remarkable adaptability to moderately warm temperatures (30°C-37°C), alkaline pH (7.0-9.0), and moderate salinity (up to 18% NaCl). These characteristics position SS15 as a promising candidate for agricultural applications, particularly in regions experiencing moderate salinity stress or alkaline soil conditions.

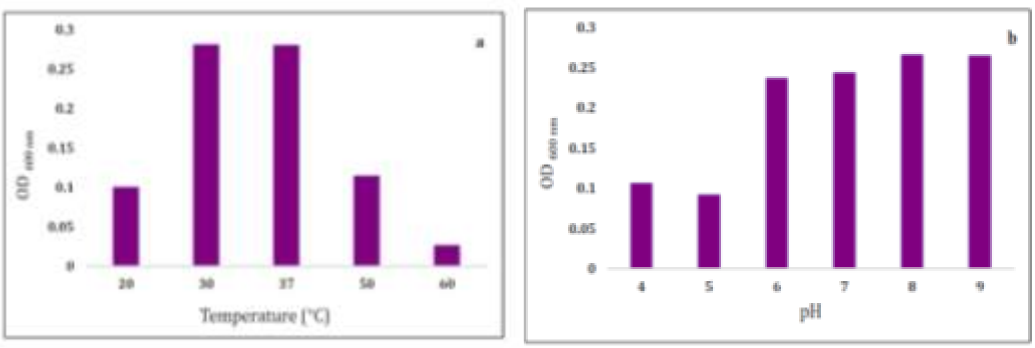

Fig 6Effect of various a) temperatures and b) pH on growth of SS15

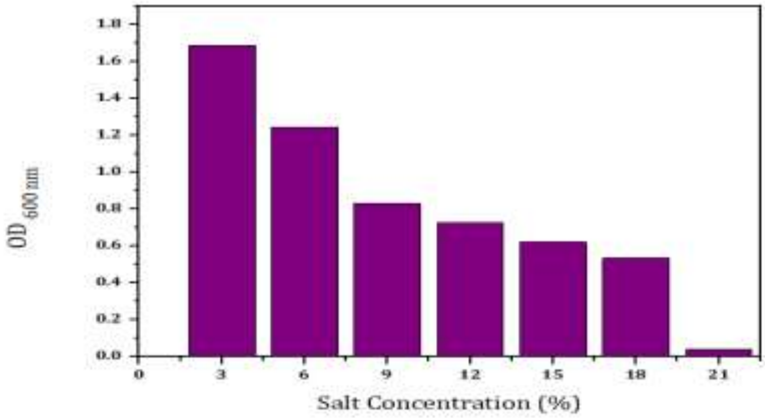

Fig 7 Effect of various salt concentration on growth of SS15

4. Discussion

4.1 Plant Growth-Promoting Mechanisms

Bacillus licheniformis SS15 demonstrated key plant growth-promoting mechanisms, including nitrogen fixation, IAA production, and phosphate solubilization. Nitrogen fixation plays a crucial role in improving soil fertility by converting atmospheric nitrogen into a form that is available to plants [2]. The production of IAA, a key phytohormone, is essential for root elongation and overall plant growth [4]. Phosphate solubilization is another important trait that aids in improving the availability of phosphorus, a vital nutrient for plant growth [3].

4.2 Environmental Tolerance

The ability of Bacillus licheniformis SS15 to grow in a broad range of pH, temperature, and salinity conditions suggests its potential for use in diverse agricultural environments. The bacterium's resilience to high salinity is particularly important in regions affected by soil salinization, a growing concern in sustainable agriculture [7].

4.3 Application in Sustainable Agriculture

The results of this study highlight the potential of Bacillus licheniformis SS15 as a biofertilizer and biocontrol agent in sustainable agriculture. Its ability to enhance nutrient availability, promote plant growth, and tolerate environmental stresses makes it a promising candidate for use in various agricultural systems.

4.4 Antagonistic Properties

The antagonistic activity exhibited by Bacillus licheniformis SS15 against common plant pathogens supports its potential as a biocontrol agent. The bacterium’s ability to produce antimicrobial compounds or directly outcompete pathogens for nutrients in the rhizosphere is a key factor in its biocontrol potential [13]. The dual-culture assays demonstrated that Bacillus licheniformis SS15 could effectively suppress the growth of soilborne pathogens, reducing the need for chemical pesticides.

5. Conclusion

Bacillus licheniformis SS15 exhibits a range of significant plant growth-promoting traits, including nitrogen fixation, production of indole-3-acetic acid (IAA), and phosphate solubilization, all of which contribute to improved plant growth and soil health. Additionally, its ability to tolerate various environmental stresses, such as temperature fluctuations, salinity, and pH changes, enhances its suitability for diverse agricultural conditions. These characteristics position Bacillus licheniformis SS15 as a promising candidate for integration into sustainable agricultural practices, offering a natural alternative to chemical fertilizers and pesticides. However, further field trials are essential to evaluate its long-term impact on crop yield, soil health, and overall ecosystem balance, providing a comprehensive understanding of its practical applications in agriculture.

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