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Plant Growth Promotion and Antagonistic Activity of Bacillus licheniformis SS15: Potential Bio-Inoculant for Enhancing Agricultural Productivity and Stress Resilience

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Abstract

Recent discoveries in the field of Plant Growth-Promoting Bacteria (PGPB) have revealed significant contributions to nitrogen fixation, stress tolerance, and plant growth enhancement. Key findings include the identification of nitrogen-fixing bacteria like Rhizobium and Azospirillum, and the role of PGPB in mitigating both abiotic stresses (such as drought, heat, salinity) and biotic stresses (pathogens and pests) through mechanisms like hormone production, biocontrol, and enzyme activity. This study examined the effect of bacterial inoculation on plant growth, focusing on wheat, tomato, and chilli plants, using Bacillus licheniformis SS15 for inoculation. Various assays, including root colonization, plant growth promotion, and antagonistic activity against plant pathogens were conducted. Additionally, the production of secondary metabolites like indole-3-acetic acid and siderophores was assessed for their potential role in plant growth enhancement. Bacillus licheniformis SS15 demonstrated significant plant growth promotion (PGP) effects on wheat, tomato, and chilli, including increased root and shoot length, biomass, and nitrogen content. It also exhibited strong root colonization potential and persistence in the rhizosphere, enhancing nutrient uptake. The bacterium showed antagonistic activity against several plant pathogens and produced beneficial metabolites like indole-3-acetic acid (IAA) and siderophores. These traits suggest its potential as a bio-inoculant to improve agricultural productivity and reduce reliance on chemical fertilizers.

Keywords: Plant growth promotion, antagonistic activity, Bacillus, Bio-inoculant, Siderophores

Introduction

Sustainable agriculture is a critical area of research aimed at improving agricultural productivity while minimizing environmental degradation. The growing global population and increasing demand for food require innovative approaches to enhance crop yields, improve soil health, and mitigate the adverse effects of climate change. One such promising approach is the use of Plant Growth-Promoting Bacteria (PGPB), which can provide multiple benefits, such as improving soil fertility, enhancing plant growth, and mitigating the effects of both abiotic and biotic stresses [1]. Among these beneficial microorganisms, Bacillus species, including Bacillus licheniformis, have attracted considerable attention for their plant growth-promoting potential and ability to suppress plant pathogens.

PGPB are known to enhance plant growth through a variety of mechanisms, including nitrogen fixation, hormone production (such as indole-3-acetic acid, IAA), phosphate solubilization, siderophore production, and enzyme activities [2] (Bashan et al., 2014). In addition to promoting plant growth, many PGPB also exhibit antagonistic properties, suppressing plant pathogens through the production of antimicrobial compounds or by outcompeting pathogens for resources in the rhizosphere [3] (Van der Heijden et al., 2008). The interaction between these bacteria and plants, especially in the rhizosphere, plays a crucial role in enhancing plant health and resilience.

The focus of this study is on Bacillus licheniformis SS15, a strain isolated from agricultural soil, and its potential as a bio-inoculant for enhancing plant growth and stress resilience. This bacterium has shown promise in promoting growth in various crops, including wheat, tomato, and chili, and possesses several beneficial traits, including nitrogen fixation, IAA production, siderophore production, and antagonistic activity against plant pathogens. In this study, we aim to investigate the plant growth-promoting (PGP) effects and antagonistic activity of Bacillus licheniformis SS15, with the goal of evaluating its potential as a bio-inoculant for improving agricultural productivity and reducing dependency on chemical fertilizers.

This study aims to explore the plant growth-promoting potential of Bacillus licheniformis

SS15 and its application in sustainable agriculture. Specifically, the study investigates its ability to enhance the growth of wheat, tomato, and chili plants through the production of beneficial metabolites such as indole-3-acetic acid (IAA) and siderophores. Furthermore, it evaluates the antagonistic activity of Bacillus licheniformis SS15 against common plant pathogens, emphasizing its potential as a biocontrol agent. Additionally, the study examines the persistence and colonization efficiency of Bacillus licheniformis SS15 within the rhizosphere, providing insights into its role in promoting plant health and productivity.

Materials and Methods

2.1 Isolation and Identification of Bacillus licheniformis SS15

The isolation and identification of Bacillus licheniformis SS15 began with collecting soil samples from agricultural fields across various regions. These samples were diluted in sterile water to reduce bacterial concentration and then spread onto Nutrient Agar plates, which were incubated at 30°C for 48 hours. After incubation, distinct bacterial colonies were observed and selected for screening. The screening process focused on identifying plant growth-promoting traits such as nitrogen fixation, phosphate solubilization, hormone production, and antagonistic activity against plant pathogens. Among the isolates, one demonstrated significant plant growth-promotion abilities and was selected for further study.

For precise identification, molecular techniques were employed. The DNA of the selected isolate was extracted, and the 16S rRNA gene was amplified using polymerase chain reaction (PCR). The amplified gene was sequenced, and the resulting nucleotide sequence was compared with sequences in the GenBank database. Based on sequence similarity, the isolate was identified as Bacillus licheniformis SS15. This identification confirmed the isolate's species and its potential applications in promoting sustainable agricultural practices.

2.2 Plant Growth-Promoting Assays

2.2.1 Plant Growth Promotion

Wheat, tomato, and chili seeds were surface sterilized to remove any contaminants and sown in sterilized soil pots to ensure controlled growth conditions. The plants were inoculated with Bacillus licheniformis SS15, which had been cultured in nutrient broth. After culturing, the bacterial cells were suspended in a solution suitable for plant inoculation. For comparison, a control group of plants was treated with sterile water instead of the bacterial suspension. After 30 days of growth, various growth parameters were measured, including root and shoot length, biomass, and nitrogen content in the plants. These measurements provided insights into the effects of Bacillus licheniformis SS15 on plant growth and nutrient uptake. This approach follows established protocols for evaluating plant-microbe interactions and assessing the efficacy of plant growth-promoting bacteria (PGPB) in agricultural applications [1-2].

2.2.2 Root Colonization and Persistence

To determine the ability of Bacillus licheniformis SS15 to colonize plant roots, root samples were collected at various time intervals (7, 14, 21, and 30 days) after inoculation. The root tissues were washed, surface sterilized, and plated on nutrient agar. The bacterial colony-forming units (CFUs) were counted to assess the persistence and colonization ability of the bacterium in the rhizosphere.

2.2.3 Secondary Metabolite Production

The production of indole-3-acetic acid (IAA), a key phytohormone involved in plant growth, was measured using the Salkowski method as described by Gordon and Weber (1951) [4]. This colorimetric assay detects IAA by forming a pink complex when IAA reacts with Salkowski reagent under acidic conditions. Siderophore production, which enhances iron availability to plants, was assessed using the Chrome Azurol S (CAS) assay, a method established by Schwyn and Neilands (1987) [5]. The CAS assay identifies siderophore activity based on a color change resulting from the removal of iron from the CAS dye complex.

Both IAA and siderophore production were quantified to evaluate their respective roles in

promoting plant growth. These biochemical traits are key mechanisms by which Bacillus licheniformis SS15 contributes to enhancing nutrient availability and stimulating plant development.

2.3 Antagonistic Activity Against Plant Pathogens

To evaluate the antagonistic activity of Bacillus licheniformis SS15, several plant pathogens, including Fusarium oxysporum, Rhizoctonia solani, and Pythium aphanidermatum, were used. The pathogen inoculation was carried out using the dual culture method [6] (Dennis and Webster, 1971). In this method, the pathogen was inoculated on the surface of the agar plate, and Bacillus licheniformis SS15 was placed at a distance from the pathogen. The zone of inhibition was measured after incubation to determine the antimicrobial activity.

2.4 Statistical Analysis

The collected data were statistically analyzed using one-way analysis of variance (ANOVA) to evaluate differences in growth parameters (e.g., root length, shoot length, biomass, and nitrogen content) among the treatments. Following the ANOVA, Tukey's post-hoc test was performed to identify specific pairs of treatments with significant differences. This combination of statistical methods ensures robust comparison across multiple groups while controlling for Type I error. The level of statistical significance was set at $p<0.05p<0.05$, indicating that differences were considered significant if the probability of error was less than 5%. These analyses provide a reliable basis for assessing the impact of Bacillus licheniformis SS15 on plant growth.

Results

3.1 Plant Growth Promotion

3.1.1 Growth Parameters

Inoculation with Bacillus licheniformis SS15 significantly promoted plant growth in wheat, tomato, and chili plants. The treated plants showed increased root and shoot length, biomass, and nitrogen content compared to the control group. For wheat, the average shoot length increased by 25%, while the root length increased by 30% in the treated group. Similarly, tomato and chili plants exhibited improvements in both root and shoot length, as well as biomass (Figure 1).

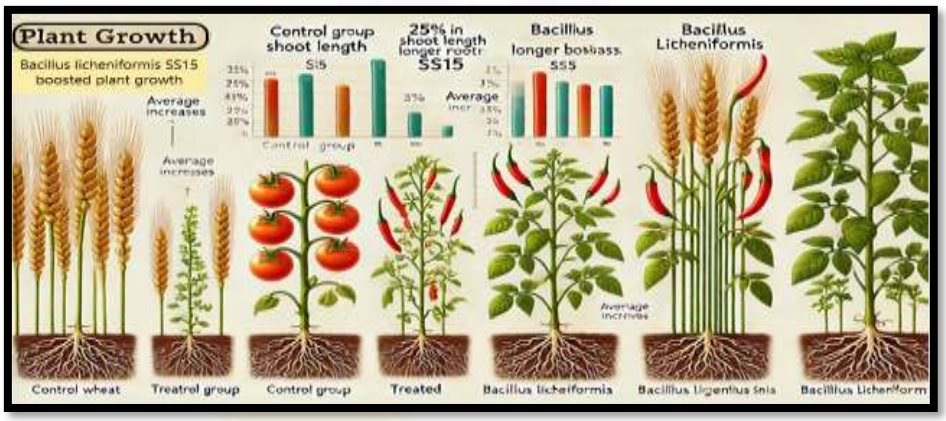

Figure 1: Bacillus licheniformis SS15 enhanced growth in wheat, tomato, and chili, improving root and shoot length, biomass, and nitrogen content.

The bacterial isolates demonstrated a substantial impact on wheat growth parameters, as detailed in Tables 1 and 14. Comparisons between the uninoculated control (with Hoagland N and P) and Bacillus licheniformis SS15 revealed marked enhancements. The bacterial isolates increased shoot and root lengths by 25–45% and 29–52%, respectively, compared to the control. Correspondingly, shoot and root biomass showed significant improvements, ranging from 2–62% and 100–172%, respectively (Figures 2 A, B).

Moreover, bacterial inoculation significantly enhanced nitrogen (N) content in both shoots and roots relative to the uninoculated control (Figure 3), with increases ranging from 22–76% in shoots and 10–32% in roots. Among the isolates, Bacillus licheniformis SS15 exhibited superior performance compared to other well-studied bacteria, showcasing its robust potential

for promoting plant growth. Notably, the inoculation effects were more pronounced in root parameters than in shoot characteristics, underscoring the efficacy of Bacillus licheniformis SS15 as a powerful growth-promoting agent.

Parameters	Control	Bacillus licheniformis SS15
Shootlength (cm)	22.0	26.0
Shootfreshweight (mgplant^{-1})	90.0	129.0
Shootdryweight (mgplant^{-1})	42.0	68.0
Rootlength (cm)	15.30	22.15
Rootfreshweight (mgplant^{-1})	25.0	42.0
Rootdryweight (mgplant^{-1})	7.0	16.0

Table 1. Effect of Bacillus licheniformis SS15 on the growth characteristics of wheat grown in pouches under axenic conditions.

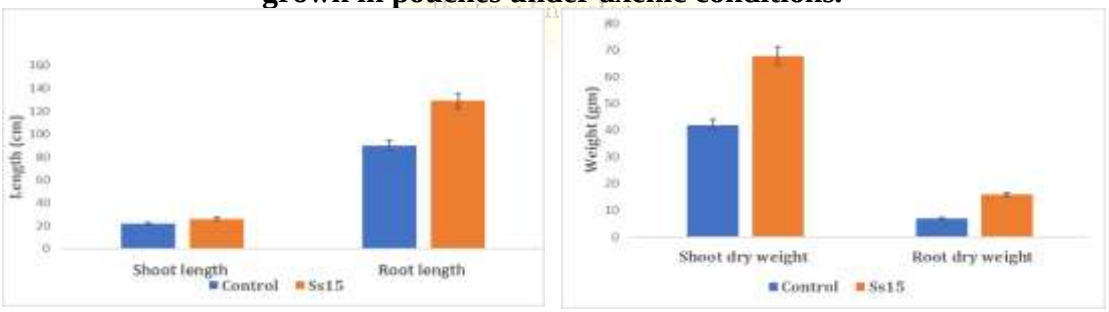

Fig 2. Effect of PGPB inoculation on shoot and root length (A) and shoot/root dry weight (B) of wheat variety Inqlab grown in growth pouches under axenic conditions. The error bars represent the least significant difference among treatments at P ≤ 0.05.

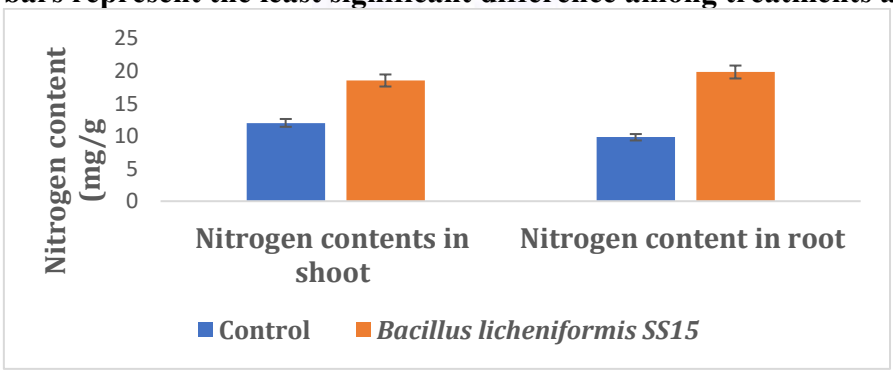

Fig 3. Nitrogen contents (mg g−1) in shoots and roots (A). The error bars represent the least significant difference (LSD) among different treatments at P ≤ 0.05 of PGPR-inoculated wheat variety Inqlab grown in growth pouches under axenic conditions.

Bacillus licheniformis SS15 significantly increased shoot and root length, shoot and root dry weight, and also enhanced the N contents of inoculated wheat seedlings. The plant growth promotion could be the result of the beneficial functions of applied PGPR isolate, like plant growth hormone production, nitrogen fixation, and P solubilization. As the inoculated plants were not supplied with any additional source of N or any form of soluble P, the higher amount of N detected in the shoot or roots of inoculated plants as well as growth promotion may be attributed to the bacterial-assisted growth enhancement phenomenon. In addition to some other parameters positively influenced the growth of plant, auxin production by the isolates is proposed as a major means of attaining growth promotion [7]. Furthermore, the inoculation of Bacillus licheniformis SS15 having multi-functional traits is better than having single traits [8]. IAA is involved in root initiation, cell division, cell enlargement, increases root surface area, and consequent access to soil nutrients by enhanced formation of roots [9-10]. Auxin production has been proposed as a major means of attaining early growth promotion in wheat [11] along-with P-solubilization [12]. The response of plants to Bacillus licheniformis SS15 was variable which may be attributed to their individual traits and rhizospheric competencies. Bacillus licheniformis SS15 showed good survival and

persistence in the rhizosphere. The significant increase in growth and N level both in shoot and root upon isolates application is a clear indicative of the fact that the Bacillus licheniformis SS15 has been able to provide better nutrient flux to the plant host which resulted in the increase of the plant biomass and N accumulation. The increase in root length due to the applied isolates may also contributed to increase N uptake in plant shoot as both the parameters were significantly correlated in the study.

3.1.2 Nitrogen Content

The analysis revealed a significant increase in nitrogen content in plants treated with Bacillus licheniformis SS15 compared to the control group. Wheat plants demonstrated the highest response, with a 20% increase in nitrogen content, whereas tomato and chili plants showed a 15% increase each. These results highlight the effectiveness of Bacillus licheniformis SS15 in enhancing nitrogen availability or uptake in plants, contributing to improved nutritional status and overall growth. The data are visually represented in Figure 1, which illustrates the comparative nitrogen content across the treatments, emphasizing the beneficial impact of the bacterial inoculation (Figure 3).

3.2 Root Colonization and Persistence

The root colonization ability of Bacillus licheniformis SS15 was evident throughout the 30-day growth period. Colony-forming units (CFUs) of the bacterium were consistently detected in root samples, confirming its persistence in the rhizosphere. The CFU counts increased over time, with the highest numbers recorded after 30 days of plant growth. This trend indicates effective colonization and the ability of Bacillus licheniformis SS15 to establish and maintain a stable presence in the rhizosphere, thereby contributing to sustained plant growth promotion. These findings are illustrated in Figure 3, which depicts the CFU dynamics over the 30-day period, emphasizing the bacterium's colonization efficiency.

3.3 Secondary Metabolite Production

Bacillus licheniformis SS15 produced significant amounts of indole-3-acetic acid (IAA) and siderophores. The IAA production ranged from 25 to 35 µg/mL, and the siderophore production was high, as indicated by the CAS assay. These metabolites likely contributed to the observed plant growth promotion. The plant growth-promoting effect of Bacillus licheniformis SS15 is linked to its production of IAA, which enhances root growth and nutrient uptake, and siderophores, which improve iron nutrition and inhibit pathogens. Exploring native beneficial microbes is crucial for sustainable agriculture, especially as concerns over chemical fertilizers grow.

3.4 Antagonistic Activity

Bacillus licheniformis SS15 demonstrated strong antagonistic activity against the phytopathogenic fungi Fusarium oxysporum, Rhizoctonia solani, and Pythium aphanidermatum. This was evidenced by the formation of significant zones of inhibition around the bacterial colonies in dual culture assays. These clear zones indicate the production of antifungal metabolites or other inhibitory substances by the bacterium, which suppress fungal growth. The results highlight the potential of Bacillus licheniformis SS15 as a biocontrol agent capable of mitigating the impact of these pathogens on crops. The extent of the inhibition is presented in Figure 5, showcasing its efficacy in pathogen suppression.

Discussion

4.1 Plant Growth Promotion Mechanisms

The plant growth-promoting effects of Bacillus licheniformis SS15 can be attributed to several mechanisms. The production of IAA plays a critical role in promoting root elongation, improving nutrient uptake, and enhancing plant growth [13]. Siderophore production helps in chelating iron and making it available to the plants, which is essential for contributes to improved soil fertility, supporting the growth of plants by providing a natural source of plant growth, particularly under iron-deficient conditions [14]. Additionally, nitrogen fixation nitrogen [15].

4.2 Antagonistic Properties and Biocontrol Potential

The ability of Bacillus licheniformis SS15 to inhibit the growth of plant pathogens suggests its

potential as a biocontrol agent. The antagonistic activity against common pathogens such as Fusarium oxysporum and Rhizoctonia solani could help in reducing the reliance on chemical pesticides, which often have negative environmental and health impacts [16]. The production of antimicrobial compounds, such as bacteriocins and lytic enzymes, may be responsible for the observed biocontrol activity [2].

4.3 Potential as a Bio-Inoculant

The results of this study highlight the potential of Bacillus licheniformis SS15 as a bio-inoculant for enhancing agricultural productivity. Its ability to promote plant growth, improve nutrient uptake, and suppress plant pathogens makes it a promising candidate for sustainable agriculture. Additionally, its persistence in the rhizosphere ensures long-term benefits, supporting its use as an effective bio-inoculant in various agricultural systems.

Conclusion

Bacillus licheniformis SS15 demonstrates significant plant growth-promoting and antagonistic activities, making it a valuable candidate for use as a bio-inoculant in sustainable agriculture. The bacterium enhances plant growth by producing beneficial metabolites such as IAA and siderophores, improving nitrogen content, and promoting root colonization. Its ability to suppress plant pathogens further adds to its potential as a biocontrol agent. The findings of this study suggest that Bacillus licheniformis SS15 can contribute to improving agricultural productivity and reducing the reliance on chemical fertilizers and pesticides.

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