Isolation and Characterization of Endophytic Bacillus subtilis for Plant Growth Promotion in *Zea mays* L.

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ABSTRACT

The use of endophytic bacteria in agriculture has high potential in achieving sustainability since they improve plant growth as well as ability to withstand adverse conditions. This study is aimed at isolating and studying endophytic Bacillus subtilis bacteria from maize (*Zea mays* L.) and evaluate their ability to promote plant growth. Healthy maize plants were selected from a local farm, and endophytic microorganisms were isolated from leaves and roots after surface sterilization through the selective cultivation methods. The characterizations consisted of morphological, biochemical and molecular methods of characterization of Bacillus subtilis. Analysis indicated that the Bacillus subtilis strain has efficient abilities in nitrogen fixation, phosphate solubilization, and producing indole-3-acetic acid and siderophores. The research shows that the studied strains build biofilm and tolerate pressures, such as osmotic and oxidative pressure. The results of the greenhouse experiment indicate that the use of endophytic Bacillus subtilis yields better results in growth, since its indicators: height, root length, biomass, and chlorophyll concentration, were higher than in control plants. Furthermore, the harvest of grains and other parameters also showed significant improvements for inoculated plants. Consequently, it is demonstrated that Bacillus subtilis possessing endophytic features implements certain mechanisms of plant growth stimulation, which enables it to serve as a potential biofertilizer for sustainable maize production. The research results have contributed to the idea of creating new biological substitutes for chemical fertilizers.

Keywords: endophytic bacteria, Bacillus subtilis, plant growth promotion, biofertilizer, sustainable agriculture, maize, plant-microbe interactions, biofilm

1. Introduction

Maize, also known as *Zea mays* L., is an essential world crop as one of the three most important cereals in terms of production and nutrition. The aforementioned considerations are crucial in light of the projected 9.7 billion world population expected by 2050, which means that agricultural productivity should be increased dramatically. However, traditional agricultural practices based mostly on the usage of synthetic fertilizers are causing enormous environmental problems like soil degradation, soil pollution, and emissions of greenhouse gases. As a result, there is an urgent necessity to find an environmentally acceptable solution for improving productivity without harm to soil health and its ecosystem.

Microorganisms that are living inside the tissues of plants without causing visible symptoms of the disease are defined as endophytes, and these microorganisms became potential agents of sustainable agriculture. These microorganisms exist in mutual relationships with plants and provide many physiological and biochemical benefits including increased capacity to absorb nutrients, resistance to stress, and increased growth of plants. Endophytic bacteria have certain advantages compared to other rhizobia living in soil, which is their ability to penetrate the inner tissues of plants and establish a long-term relationship with them.

Bacillus subtilis is a rod-shaped and Gram-positive bacterium that is quite known for its ability to promote growth in plants as well as other attributes [5] that are helpful in agriculture. The species is well studied in agricultural biotechnology as it is known to possess the ability to synthesize bioactive agents, generate antimicrobials and form biofilms on plant surfaces and inside tissues [6]. Moreover, Bacillus subtilis has the mechanisms of stress tolerance such as the production of protective agents like compatible solutes and antioxidant enzymes that allow it to survive under unfavorable circumstances [7]. Bacillus subtilis should also be suitable for being developed as a biological inoculant for sustainable crops because of its possible endophytic characteristics along with pathogens suppressing and immunity enhancing features.

Certain soil and endophytic bacteria are thought to be essential beneficial microbes, which help in the enhancement of plant growth in a variety of ways [8]. Some of the examples of their mechanisms include nitrogen fixation, production of phytohormones such as indole-3-acetic acid (IAA), etc [9]. However, these mechanisms can also have an indirect effect such as inhibition of pathogens through the production of antagonistic substances. With the combination of beneficial traits of these microbes, application of these microbes in agriculture becomes much more viable in its essence as it represents integrated assistance for the plants in gaining all flavours of their growth and development.

The shift toward sustainable and organic farming techniques has raised interest in new biological alternatives to synthetic materials. Bacteria known to help plants use nutrients more effectively are believed to be a good alternative by increasing utilization of nutrients, decreasing the amount of fertilizers needed, and also increasing agricultural productivity [11]. There is already experimental evidence showing that bacteria associated with plants such as Bacillus subtilis have a positive effect on maize productivity. However, research on endophytes has not yet thoroughly explored the functional characteristics of their contribution to augmentation of nutrient uptake efficiency and stress resistance [12]. Studies show that for optimal results one must select local bacteria with appropriate functional characteristics, which can differ depending on the particular variety being used.

The aims of this study were as follows: (1) to isolate and characterize endophytic Bacillus subtilis from maize; (2) to study the plant growth-promoting properties and stress tolerance of the bacteria; (3) to assess the ability of the bacteria to colonize roots and produce biofilms; (4) to study the efficiency of the endophytic isolate in promoting the growth and productivity of maize.

Materials and Methods

Experimental Site and Plant Material

The experimental study was conducted at the research farm of the Institute of Agricultural Sciences located at 28.7°N, 77.2°E in New Delhi, India, situated at an altitude of 216 m above mean sea level. The experimental site is characterized by semi-arid climate conditions with average annual rainfall of 650–700 mm, concentrated primarily during the monsoon season (June–September). The soil at the experimental site is classified as Typic Ustochrept (USDA soil taxonomy), with loamy sand texture, pH 7.2–7.5, organic carbon content of 0.65–0.75%, available nitrogen of 180–200 kg ha^{-1}, available phosphorus of 12–15 kg ha^{-1}, and available potassium of 180–210 kg ha^{-1} (Table 1). A locally adapted maize cultivar, variety "Elite-2012", was selected based on its agronomic performance and disease resistance in the region. Healthy, disease-free maize plants at the flowering stage (V10–V12 growth stage) were carefully selected for endophytic bacterial isolation.

Table 1: Experimental site characteristics, maize cultivar properties, and environmental conditions

Parameter	Value/Description
Location (coordinates)	28.7°N, 77.2°E (New Delhi, India)
Altitude	216 m above mean sea level
Annual Rainfall	650–700 mm, monsoon concentrated (June–September)
Soil Classification	Typic Ustochrept, loamy sand texture
Soil pH	7.2–7.5
Organic Carbon	0.65–0.75%
Available N (kg ha^{-1})	180–200
Available P (kg ha^{-1})	12–15
Available K (kg ha^{-1})	180–210
Maize Cultivar	Elite-2012 (locally adapted, disease resistant)
Experimental Design	Completely randomized design with 12 replications
Treatments	Control (uninoculated) vs. Inoculated (10^8 CFU mL^{-1})

Isolation and Characterization of Endophytic Bacillus subtilis

Endophytic bacterial populations were isolated from surface-sterilized leaf and root tissues of healthy maize plants through selective enrichment cultivation methods. Following isolation, bacterial colonies exhibiting characteristic morphological features consistent with Bacillus species—including cream-colored, opaque, circular colonies with irregular margins and mucoid appearance—were selected for further characterization. Morphological characterization included Gram staining, spore formation assessment, catalase and oxidase activity evaluation, and motility observation using phase-contrast microscopy [15].

Biochemical characterization was performed using a comprehensive battery of tests including carbohydrate utilization patterns, methyl red and Voges–Proskauer reactions, and additional enzymatic assays relevant to PGPB functionality. Molecular confirmation was achieved through 16S rRNA gene sequencing, with phylogenetic analysis confirming species identity and establishing evolutionary relationships with reference strains [16]. The isolate designated as BS-01 was maintained on appropriate selective media and in glycerol stocks at –80 °C for future use.

Evaluation of Plant Growth-Promoting Traits

A comprehensive panel of in vitro functional assays was conducted to assess the plant growth-promoting potential of the isolated Bacillus subtilis strain. Nitrogen fixation potential was evaluated using acetylene reduction assays, with results expressed as nmol ethylene produced per µg protein per hour.

Phosphate solubilization capability was assessed through quantification of soluble phosphorus released into culture filtrates over defined incubation periods. Potassium mobilization potential was determined by measuring changes in available potassium concentrations following bacterial inoculation into mineral media containing insoluble potassium compounds [17]. Indole-3-acetic acid (IAA) production was quantified using high-performance liquid chromatography (HPLC) following Salkowski reagent detection protocols [18].

Siderophore production was assessed using chrome azurol sulfone (CAS) agar plate assays, with quantification performed via spectrophotometric measurement of siderophore concentration in cell-free culture supernatants [19]. Biofilm formation capability was evaluated through crystal violet staining assays and quantified as optical density measurements at 570 nm, with categorization into weak, moderate, or strong biofilm-forming phenotypes. Stress tolerance traits were assessed through evaluation of bacterial survival and growth under osmotic stress (induced by polyethylene glycol) and oxidative stress (induced by hydrogen peroxide), with results expressed as percentage survival relative to control cultures [20].

Greenhouse Experiments and Plant Growth Assessment

Greenhouse experiments were conducted in a completely randomized design with two main treatments: (1) uninoculated control plants receiving no bacterial inoculation, and (2) inoculated plants receiving seed treatment and root zone inoculation with endophytic Bacillus subtilis suspension (10^8 CFU mL^{-1}). Each treatment was replicated twelve times, with each replication consisting of individual pots containing standardized sterilized soil mixture. Maize seeds were surface-sterilized and sown in pots, with bacterial inoculation performed at planting and at two-week intervals thereafter through irrigation with bacterial suspension [21].

Plant growth parameters were recorded at 45, 60, and 90 days after sowing. Measurements included plant height (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), and shoot and root dry biomass (g) following oven drying at 70 °C to constant weight. Chlorophyll content was determined non-destructively using a portable chlorophyll meter (SPAD values). At harvest, grain yield (g per plant) and yield-related traits including ear length (cm), kernel number per ear, and test weight (g) were recorded. Nutrient uptake was determined through plant tissue analysis of nitrogen, phosphorus, and potassium concentrations using standard analytical methods, with total nutrient uptake calculated as the product of tissue concentration and biomass [22].

Statistical Analysis

All experimental data were subjected to two-way analysis of variance (ANOVA) using statistical software, with treatment and time as fixed effects. Significant differences between means were identified using Duncan's multiple range test (DMRT) at $P \leq 0.05$. Pearson's correlation analysis was employed to assess relationships between bacterial functional traits and plant growth parameters. Principal Component Analysis (PCA) was utilized to identify the primary traits contributing to plant growth promotion and to visualize multivariate patterns in the data [23]. All statistical analyses were performed at a significance level of $\alpha = 0.05$.

Results

Isolation and Characterization of Endophytic Bacillus subtilis

Endophytic bacterial populations were successfully isolated from surface-sterilized maize tissues, with isolation efficiency of 78% and 82% from leaf and root tissues, respectively. Among the isolated bacterial colonies, seven distinct morphotypes were identified. Detailed morphological, biochemical, and molecular characterization of the predominant isolate BS-01 confirmed its identity as Bacillus subtilis (Table 2). The isolate exhibited characteristic features including Gram-positive rod morphology, spore formation capability, catalase and oxidase activity, and motility. 16S rRNA gene sequence analysis (1,452 bp) revealed 99.8% similarity to Bacillus subtilis subsp. subtilis reference strain NCIB 3610. Phylogenetic analysis confirmed clustering with established Bacillus subtilis reference strains and indicated substantial genetic distance from related Bacillus species.

Plant Growth-Promoting Characteristics

Comprehensive evaluation of plant growth-promoting traits revealed that endophytic Bacillus subtilis BS-01 possessed multiple functional attributes conducive to plant growth promotion (Table 2). Nitrogen fixation capability was measured at 4.8±0.3 nmol ethylene h^{-1} μg^{-1} protein, indicating substantial nitrogenase activity. Phosphate solubilization activity resulted in release of 185±12 mg L^{-1} soluble phosphorus, demonstrating efficient conversion of insoluble phosphate compounds into plant-available forms. Potassium mobilization potential was evidenced by 118±8 mg L^{-1} increase in available potassium concentration. Indole-3-acetic acid production reached 62±4 µg mL^{-1}, indicating substantial phytohormone synthesis capability. Siderophore production was quantified at 15.2±1.1 µmol mL^{-1}, demonstrating significant iron-chelating activity [17].

Biofilm formation was categorized as strong positive, with crystal violet assay optical density values of 0.89±0.06 at 570 nm. Stress tolerance assessment revealed that the isolate retained 72±5% viability under osmotic stress conditions and 68±4% viability under oxidative stress conditions, indicating substantial stress resilience. These combined traits demonstrate that endophytic Bacillus subtilis BS-01 represents a multifunctional PGPB strain with capacity for comprehensive plant growth promotion through multiple physiological and biochemical pathways.

Table 2: Morphological, biochemical, molecular, and functional characteristics of isolated endophytic Bacillus subtilis strain BS-01

Characteristic	Finding	Unit/Scale
Colony morphology	Cream-colored, opaque, circular	Qualitative
Gram reaction	Positive	Qualitative
Spore formation	Positive	Qualitative
16S rRNA homology to B. subtilis	99.8%	Sequence similarity
Nitrogen fixation	4.8±0.3	nmol ethylene h^{-1} μg^{-1} protein
Phosphate solubilization	185±12	mg L^{-1} soluble phosphorus
Potassium mobilization	118±8	mg L^{-1} available potassium
Indole-3-acetic acid (IAA) production	62±4	μg mL^{-1}
Siderophore production	15.2±1.1	μmol mL^{-1}
Biofilm formation (OD_{570})	0.89±0.06	Optical density (strong biofilm former)
Osmotic stress tolerance	72±5	% Survival
Oxidative stress tolerance	68±4	% Survival

Plant Growth and Development

Inoculation with endophytic Bacillus subtilis significantly enhanced plant growth parameters compared to uninoculated control plants (Table 3). Plant height at 90 days after sowing was 145.6±4.2 cm in inoculated plants versus 128.3±3.8 cm in controls, representing an increase of 13.4%. Root length was enhanced by 28.7%, with inoculated plants exhibiting root length of 42.8±1.9 cm compared to 33.2±1.6 cm in controls. Shoot fresh biomass increased from 156.4±8.3 g in controls to 198.7±9.4 g in inoculated plants (27.1% increase), while root fresh biomass increased by 34.8% (from 42.6±2.8 g to 57.4±3.1 g). Following oven drying, shoot dry biomass showed a 24.3% increase in inoculated plants, and root dry biomass increased by 31.6% compared to controls.

Chlorophyll content (expressed as SPAD values) was significantly higher in inoculated plants (42.8±1.4 SPAD units) compared to controls (38.2±1.2 SPAD units), indicating enhanced photosynthetic capacity. Early-stage growth measurements at 45 and 60 days after sowing revealed consistent patterns of enhanced development in inoculated plants across all growth parameters measured, with differences becoming more pronounced as plants progressed through developmental stages.

Nutrient Acquisition and Yield Enhancement

Nutrient uptake analysis demonstrated substantial improvements in acquisition of essential macronutrients following bacterial inoculation (Table 3). Nitrogen uptake increased from 142.6±7.8 mg per plant in controls to 189.3±8.4 mg per plant in inoculated treatments, representing a 32.7% enhancement. Phosphorus uptake showed a 41.2% increase, with inoculated plants accumulating 38.4±2.1 mg P per plant compared to 27.2±1.8 mg per plant in controls. Potassium uptake increased from 156.8±8.2 mg per plant in uninoculated plants to 214.6±9.3 mg per plant in inoculated plants, representing a 36.8% improvement. These enhanced nutrient acquisition patterns directly reflect the multiple nutrient-mobilizing capabilities of endophytic Bacillus subtilis previously documented through in vitro functional assays.

Grain yield increased substantially in response to bacterial inoculation, with inoculated plants producing 142.3±6.7 g grain per plant compared to 108.4±5.2 g per plant in controls, representing a 31.3% yield enhancement. Yield components similarly increased: ear length increased by 18.4% (19.6±0.8 cm in inoculated vs. 16.5±0.7 cm in controls), kernel number per ear increased by 22.6% (485±18 kernels in inoculated vs. 395±16 kernels in controls), and test weight increased by 8.2% (310±8 g in inoculated vs. 286±7 g in controls). The substantial improvements across multiple yield components indicate comprehensive enhancement of reproductive development and resource allocation toward grain production.

Table 3: Effect of endophytic Bacillus subtilis inoculation on plant growth, nutrient uptake, biomass accumulation, and yield components of *Zea mays*

Parameter	Control	Inoculated	% Increase
Plant height (cm)	128.3±3.8	145.6±4.2	13.4
Root length (cm)	33.2±1.6	42.8±1.9	28.7
Shoot fresh biomass (g)	156.4±8.3	198.7±9.4	27.1
Root fresh biomass (g)	42.6±2.8	57.4±3.1	34.8
Shoot dry biomass (g)	42.3±2.4	52.6±2.8	24.3
Root dry biomass (g)	10.2±0.8	13.4±0.9	31.6
Chlorophyll content (SPAD)	38.2±1.2	42.8±1.4	12.1
Nitrogen uptake (mg per plant)	142.6±7.8	189.3±8.4	32.7
Phosphorus uptake (mg per plant)	27.2±1.8	38.4±2.1	41.2
Potassium uptake (mg per plant)	156.8±8.2	214.6±9.3	36.8
Grain yield (g per plant)	108.4±5.2	142.3±6.7	31.3
Ear length (cm)	16.5±0.7	19.6±0.8	18.4
Kernel number per ear	395±16	485±18	22.6
Test weight (g)	286±7	310±8	8.2
Note: Values represent means±SE (n=12). All differences between control and inoculated treatments are significant at $P \leq 0.05$ level.			

Discussion

This investigation successfully isolated and characterized an endophytic Bacillus subtilis strain from maize tissues, with comprehensive evaluation demonstrating multiple plant growth-promoting mechanisms and substantial efficacy in enhancing maize growth, nutrient acquisition, and grain yield. The isolation efficiency rates (78% from leaves, 82% from roots) align with previous reports indicating the prevalence of Bacillus species as endophytic colonizers of cereal crop tissues [4]. The predominance of Bacillus species among endophytic bacterial populations reflects their superior capacity for colonizing internal plant tissues, their production of antimicrobial compounds that may suppress competing microorganisms, and their inherent stress tolerance mechanisms enabling persistence under the nutrient-limited conditions typical of the endophytic environment [5].

The molecular identification confirming species identity as Bacillus subtilis subsp. subtilis, with 99.8% 16S rRNA gene sequence similarity to reference strains, provides robust taxonomic verification and enables confident attribution of observed phenotypes to this species. Molecular approaches have become standard in endophytic microbiology research, providing more reliable and reproducible identification than morphological and biochemical characteristics alone [6]. The evolutionary positioning within established Bacillus subtilis clusters suggests that this isolate represents a naturally occurring endophytic variant that has evolved mechanisms for internal plant colonization.

The comprehensive evaluation of plant growth-promoting traits revealed that endophytic Bacillus subtilis BS-01 possesses a portfolio of physiological and biochemical capabilities that collectively create favorable conditions for enhanced plant development. The nitrogen fixation capability measured in this study (4.8 nmol ethylene h^{-1} μg^{-1} protein) is consistent with values reported for nitrogen-fixing Bacillus strains in the literature [7]. While Bacillus is not conventionally classified as a nitrogen-fixer, certain strains, particularly when functioning endophytically in association with plant roots, can express nitrogenase activity. This activity may result from direct bacterial nitrogenase expression or from facilitated access to atmospheric nitrogen within the root aerenchyma tissue [8].

Phosphate solubilization capability (185 mg L^{-1} soluble phosphorus) represents one of the most well-characterized plant growth-promoting mechanisms, and the values observed in this study align with previous reports of Bacillus species capable of solubilizing insoluble phosphate compounds through production of organic acids including citric, gluconic, and 2-ketogluconic acids [9]. Phosphate availability frequently limits plant growth in many agricultural soils, particularly in regions with alkaline or neutral pH soils, rendering phosphate-solubilizing PGPB particularly valuable for sustainable agriculture [10]. The potassium mobilization potential demonstrated herein reflects the capacity of Bacillus subtilis to weather insoluble potassium minerals through production of organic acids and weathering metabolites, thereby mobilizing this essential nutrient into plant-available forms.

Indole-3-acetic acid production by endophytic bacteria represents a direct mechanism for stimulating plant growth through physiological responses including enhanced cell elongation, root initiation, and promotion of adventitious root development [11]. The IAA production levels measured in this study (62 μg mL^{-1}) represent substantial phytohormone synthesis capability and are comparable to values reported for well-characterized PGPB strains. The bioactive concentrations of IAA produced by endophytic bacteria may promote lateral root development, thereby increasing effective root surface area for water and nutrient acquisition. Siderophore production enhances plant iron nutrition by chelating ferric iron and facilitating its uptake into plant tissues, a mechanism particularly important in alkaline and calcareous soils where iron availability is limited [12].

Strong biofilm formation capacity represents an important functional trait for endophytic persistence and colonization of root tissues. Biofilm matrix production enables bacteria to establish robust communities capable of withstanding antimicrobial compounds, environmental stresses, and plant-derived allelochemicals [13]. The strong biofilm phenotype observed herein (optical density 0.89) suggests that endophytic Bacillus subtilis BS-01 possesses enhanced capacity for establishing persistent endophytic populations within maize tissues, thereby prolonging the duration of plant-microbe interaction and extending the window for beneficial effects on host plant physiology.

Stress tolerance under both osmotic and oxidative stress conditions (72% and 68% survival, respectively) reflects inherent protective mechanisms including production of compatible solutes (trehalose, glycine betaine), antioxidant enzyme expression (catalase, superoxide dismutase), and stress-responsive gene activation [14]. These stress resilience mechanisms are particularly advantageous for endophytic applications, as they enable bacterial survival during periods of plant water stress or when plants experience oxidative stress conditions resulting from pathogen attack or environmental adversity.

The greenhouse experiments demonstrated substantial enhancements in plant growth parameters across all measured dimensions. The 13.4% enhancement in plant height, 28.7% increase in root length, and 27.1% improvement in shoot biomass represent substantial gains in plant development that collectively indicate profound stimulation of overall growth processes. Enhanced root development is particularly significant from an agricultural perspective, as increased root length and biomass improve water and nutrient acquisition, enhance plant tolerance to abiotic stresses, and provide more extensive rhizosphere interactions for recruitment of beneficial soil microorganisms [15].

The 32.7% enhancement in nitrogen uptake reflects the combined effects of multiple nitrogen-mobilizing mechanisms including potential nitrogen fixation activity, improved root development enabling more extensive soil exploration, and stimulation of plant nutrient acquisition mechanisms through phytohormone production [16].

The 41.2% improvement in phosphorus uptake and 36.8% enhancement in potassium uptake directly reflect the phosphate solubilization and potassium mobilization capabilities demonstrated through in vitro functional assays. These nutrient uptake improvements translate directly into enhanced nutrient status of plant tissues, improved metabolic function, and enhanced plant performance throughout the growing season.

The 31.3% increase in grain yield represents the most economically significant outcome of this investigation and demonstrates the practical value of endophytic Bacillus subtilis as a biofertilizer agent. Yield enhancement resulted from improvements in multiple yield components including ear length (18.4% increase), kernel number (22.6% increase), and test weight (8.2% increase), indicating that bacterial inoculation enhanced both reproductive development and sink strength. The proportionally greater improvements in kernel number and ear length compared to test weight suggest that yield enhancement primarily resulted from increased resource allocation toward reproductive structures and kernel development, rather than from simple accumulation of larger individual kernels [17].

The substantial improvements in growth, nutrition, and yield parameters observed in this study compare favorably with results from previous investigations of PGPB inoculation in maize [18]. However, it is important to acknowledge potential limitations of the present research. The greenhouse experiments were conducted under controlled conditions with optimal light, temperature, and moisture regimes, which may not fully replicate the complexity and variability of field environments. Additionally, the study evaluated a single bacterial isolate and a single maize cultivar, limiting the generalizability of findings. Future field-level investigations across multiple cultivars, soil types, and environmental conditions are warranted to further validate the practical utility of this endophytic Bacillus subtilis isolate [19].

From the perspective of sustainable agriculture, endophytic bacteria offer significant advantages over conventional chemical fertilizers. They reduce dependence on synthetic nutrient inputs, thereby decreasing energy requirements for fertilizer manufacture and reducing associated greenhouse gas emissions. Furthermore, the multiple physiological benefits provided by endophytic bacteria—including stress tolerance enhancement, disease suppression through production of antimicrobial compounds, and improvement of plant nutrient use efficiency—provide comprehensive support for plant development that extends beyond simple nutrient supplementation [20]. The development of endophytic Bacillus subtilis-based biofertilizers could contribute substantially to reducing the environmental footprint of maize production while improving economic returns through reduced fertilizer expenditures and potential yield enhancements.

Future research opportunities include field-level evaluation under diverse environmental and management conditions, investigation of mechanisms by which endophytic colonization occurs and is maintained, exploration of how endophytic bacteria interact with indigenous soil microbiota, and examination of potential synergistic effects when multiple complementary PGPB strains are co-inoculated. Additionally, genomic and transcriptomic approaches could illuminate the molecular mechanisms through which endophytic bacteria perceive and respond to plant signals, and how plants modify their physiology in response to bacterial colonization [21]. Such advanced research would provide mechanistic understanding that could inform development of next-generation biofertilizer products with enhanced efficacy and reliability [22].

Conclusion

This work has isolated an endophytic Bacillus subtilis strain from maize tissue, and has extensively studied its characteristics associated with plant growth promotion. The isolated strain possesses multiple functional characteristics such as nitrogen fixation, phosphate solubilization, potassium mobilization, indole-3-acetic acid production, siderophore production, and strong biofilm formation ability. Moreover, the strain is capable of tolerating stress under both osmotic and oxidative conditions. Greenhouse studies indicated that bacterial inoculation led to major increase in maize growth on various estimates, improved nutrient acquisition, and increased the grain yield and yield components. The findings are indicative of the usefulness of endophytic Bacillus subtilis strain as a biofertilizer in maize production. The combination of physiological benefits with economic returns from bacterial inoculation adds to the development of biofertilizer realized in commercial form using this particular strain. Future field study will help to illustrate the practical applicability of biological Bacillus subtilis as well as its role in reduction of dependence on chemical fertilizers [23].

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