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Utilization of *Stenotrophomonas koreensis* and *Bacillus amyloliquefaciens* for Improving Growth, Reducing Nitrogen Fertilization and Controlling *Bipolaris sorokiniana* in Wheat

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Abstract

Wheat (*Triticum aestivum* L.), a vital cereal, faces significant challenges from common root rot and spot blotch diseases caused by *Bipolaris sorokiniana*. This study aimed to explore the potential of plant growth promoting rhizobacteria (PGPR) to enhance wheat growth, reduce fertilizer input, and combat *Bipolaris* diseases. Two PGPR isolates, selected for their superior antagonistic properties, were identified as *Stenotrophomonas koreensis* RB11 and *Bacillus amyloliquefaciens* RB12. These PGPR strains displayed multiple plant growth promoting and biocontrol attributes, including phosphate solubilization, indole-3-acetic acid production, nitrogen fixation and antagonism against *B. sorokiniana* and other fungi. Wheat seed priming with the PGPR significantly improved germination, plant growth, nutrient content and biomass carbon accumulation in the rhizosphere soil. Importantly, the application of RB11 and RB12 allowed for a 25% and 50% reduction in nitrogen fertilizer usage, respectively, without compromising the yield. RB11 and RB12 also demonstrated potent inhibitory effects on *B. sorokiniana* conidial germination and significantly controlled common root rot and spot blotch in wheat, similar to those observed with the fungicide Protaf 250EC. Overall, this study underscores the multifaceted roles of *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 in promoting wheat growth, reducing fertilizer inputs and effectively suppressing wheat pathogens. These findings contribute to the development of PGPR-based strategies for sustainable crop production and disease control.

Keywords: antagonist; common root rot; plant growth promoting rhizobacteria; root colonization; spot blotch

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a very important cereal crop, widely cultivated and consumed across different cultures and continents. It serves as a fundamental dietary staple for around 35% of the global populace, contributing approximately 20% of the total protein and caloric intake worldwide (Farooq, 2009). In 2020, wheat cultivation spanned more land than any other crop, encompassing one-fifth of global farms (Erenstein et al., 2021). Despite its wide cultivation, wheat production needs to be increased adequately to provide sustenance for the growing populations. However, diseases are major constraints to increased wheat production. Wheat suffers from more than 100 diseases, which cause approximately 21.5% of the wheat crop losses (Singh et al., 2023).

Bipolaris sorokiniana (Sacc.) Shoemaker is an important fungal pathogen that largely affects the warmer regions and serves as a primary causal

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organism of multiple diseases, including head blight, seedling blight, foliar blight/spot blotch, common root rot and black point (Singh, 2017). Among all diseases, wheat spot blotch is recognized as the most significant, leading to substantial grain yield losses ranging from 4 to 43% in South Asia (Sharma et al., 2012). The disease also results in reduced kernels per spike and thousand-kernel weight. Common root rot, another affliction caused by *B. sorokiniana*, threatens wheat production, inducing necrotic lesions and plant mortality (Su et al., 2021). Managing *B. sorokiniana* in wheat primarily involves deploying resistant cultivars and fungicides. However, developing fully resistant varieties remains challenging, and the extent of available resistant cultivars is unknown. Chemical fungicides, though widely used, raise concerns due to their potential impact on the environment and public health (Hossain et al., 2017; Hossain and Sultana, 2020). Thus, the usage of control approaches necessitates careful consideration of potential ecological consequences.

Microbial agents for plant growth promotion and disease suppression offer a promising sustainable approach in agriculture. Identifying plant growth promoting rhizobacteria (PGPR) with biocontrol capabilities is crucial for reducing reliance on chemical fungicides and fertilizers. Certain PGPR, including *Bacillus* and *Stenotrophomonas* spp., have been recognized for inhibiting *B. sorokiniana* growth and promoting wheat growth (Yue et al., 2018; Ullah et al., 2020). These bacteria, commonly found in soil, enhance nutrient availability in the rhizosphere, making them valuable in sustainable agriculture (Islam et al., 2016). Utilizing the PGPR would be attractive in sustainable agriculture, as they can simultaneously improve plant development with reduced fertilizer application while controlling *B. sorokiniana* diseases in wheat. Thus, the present study explores the potential of PGPR to enhance plant growth and yield under nitrogen-depleted conditions and suppress spot blotch and common root diseases in wheat.

MATERIALS AND METHOD

Experimental site

The experiment was conducted from October 2021 to April 2023 in the Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh (90.25° E, 24.09° N), which lies within the agroecological zone of the Madhupur Tract (AEZ 28). Pot studies using "swallow red brown terrace soil" belong to the Salna series of the Inceptisols order. The surface soil consisting of clay is mildly acidic (pH 6.78), with a cation exchange capacity (CEC) of 6.78 meq 100 g^{-1} and electrical conductivity (EC) of 0.6 dS m^{-2}. It contains 1.08% organic carbon, 1.87% organic matter, 0.10% N, 9 ppm P and 0.20 meq 100 g^{-1} K.

Planting materials, bacterial isolates and fungal pathogen

Wheat variety BARI Gom-27 was used as the host with seeds from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Bacteria were isolated from the rhizosphere of the cucumber (*Cucumis sativus* L.). Isolation involved surface sterilization of fresh roots, pulverization and serial dilutions for bacterial culture on Luria Agar (LA) plates (Islam et al., 2016). Purified bacterial isolates were stored in a glycerol solution at -20 °C. Fungal pathogens *Rhizoctonia solani* AR-01, *Bipolaris sorokiniana* BS-1, *Sclerotium rolfsii* SR-1 and *Sclerotia sclerotiorum* PSB-1 were obtained from the Plant Pathology Laboratory, BSMRAU.

Selection of antipathogenic isolates *in vitro* against phytopathogens

Isolated bacteria were screened for antagonistic activity against the above fungal pathogens using a dual culture technique (Hossain and Sultana, 2018). From a 5-day-old culture of these pathogens, 5-mm-diameter mycelial plugs were excised and placed in the center of the Potato Dextrose Agar (PDA) plate. The overnight bacterial culture was then streak inoculated at an equal distance of 3 cm from the fungal plug. The control cultures of phytopathogen were cultivated without bacteria. After incubation at 28 °C for seven days, the inhibition of the fungal growth compared to the control was assessed (Hossain and Sultana, 2018).

Morphological and biochemical characterization of bacterial isolates

Selected bacterial antagonists underwent morphological and biochemical characterization, including colony morphology and growth pattern (Islam et al., 2016). Biochemical tests such as Gram reaction, catalase, oxidase, KOH solubility, motility, salt stress tolerance, citrate test, and Voges-Proskauer test were conducted following standard protocols (Bergey

et al., 1994; Hossain and Sultana, 2018; Masum et al., 2018).

Molecular characterization of bacterial isolates

Genomic DNA was extracted from the antagonistic isolates, and the 16S rRNA gene was amplified using primers 27F(5′-AGAGTTTGATCCTGGCTCAG-3′)/1492R (5′-GGTTACCTTGTTACGACTT) (Hossain and Sultana, 2008; Islam et al., 2016). The PCR products were sequenced, and phylogenetic trees were constructed using the neighbor-joining approach. Bootstrap replication (1000 replicates) was applied for statistical support.

Bioassays of growth-promoting plant traits

Phosphate solubilization test

For the phosphate solubilization assay, each bacterium was inoculated onto a Pikovskaya (PVK) medium (Hossain and Sultana, 2018). The plates were kept in an incubator at 28 °C for 7 days and observed for the clear zone around the colonies.

Acetylene reduction assay

Nitrogenase activity was evaluated using the acetylene reduction assay (Hossain and Sultana, 2018). Bacterial colonies were injected into a sealed 30 ml vial with nitrogen-free semi-solid medium, and incubated at 28 °C for 48 hours. After pellicle formation, 10% acetylene gas was injected, and incubation continued for 24 hours at 28±2 °C. Ethylene production was monitored using a G-300 Gas Chromatograph. The viable cell number (CFU) was calculated based on the standard curve, determining the rate of N_2 fixation as ethylene accumulation (mol C_2H_4 mg^{-1} protein h^{-1}).

Production of indole-3-acetate

To detect and quantify indole-3-acetic acid (IAA), selected bacterial strains were inoculated into Jensen's broths and incubated at 28 °C with constant shaking at 125 rpm for 48 hours (Hossain and Sultana, 2018). A 1 ml aliquot of the supernatant was combined with 2 ml of Salkowski's reagent and incubated for 20 minutes in darkness at room temperature (150 ml concentrated H_2SO_4, 250 ml H_2O, 7.5 ml 0.5 M $FeCl_3.6H_2O$). The absorbance was measured at 530 nm using a spectrophotometer, indicating a pinkish-red hue upon IAA generation. IAA concentration was calculated using a standard curve from pure IAA solutions.

Bioassays of biocontrol traits

Siderophore and hydrocyanic acid production assay

Siderophore production was qualitatively assessed by spot inoculating bacterial cultures onto nutrient agar plates, incubating them at 28 °C for 1 to 2 days. Chrome azurol S (CAS) agar medium was prepared and covered bacterial cultures grown for 48 hours with a mixture of PIPES buffer medium and CAS dye solution (Sultana and Hossain, 2022) for 20 to 24 hours at 28 °C. For assessing hydrogen cyanide (HCN) production, bacterial colonies were streaked on nutrient agar plates. Filter papers soaked in an alkaline picrate solution were placed on Petri plate lids and incubated at 28 °C for 4 to 5 days. A change from bright yellow to brick orange indicated HCN presence.

Extracellular enzyme assays

For lipase detection, overnight bacterial cultures were spot inoculated on 1% Tween-20 Luria-Bertani (LB) agar plates (Sierra, 1957). Skim milk agar plates were utilized to detect protease production (Kumar et al., 2005). LB agar plates with 1% carboxymethyl cellulose (CMC) were used for cellulase production. Bacteria were spot inoculated, and plates were incubated at 28 °C for 3 to 4 days, observing for clear zones around colonies. Congo red staining (0.1%, 15 minutes) revealed cellulase activity (1M NaCl for 15 minutes). Positive results showed yellowish zones around bacterial growth. Chitinase detection involved spot inoculating 10 µl of overnight bacterial culture on M9 agar medium supplemented with 1% (w/v) colloidal chitin, with a clear zone indicating chitinase production.

Preparation of PGPR inoculants for treating seeds

Bacteria were cultured in 200 ml yeast peptone dextrose (YPD) broth on an orbital shaker (120 rpm) for 72 hours at 28 °C. Cells were collected by centrifugation, washed twice and suspended in 0.6 ml sterilized water. After vortexing, 15 g (33 to 35 seeds) of surface-sterilized wheat seeds were soaked in the bacterial suspension and air-dried overnight at room temperature for effective seed coating. The bacterial cell count per seed was determined using the serial dilution technique. Another set of seeds soaked in water served as a control.

In vitro effect of bacteria on seed germination of wheat

To assess the effect of bacterial treatment on wheat seed germination, 200 treated and non-treated seeds were plated onto two layered moistened filter papers in 9-cm petri dishes, with 25 seeds per plate. The dishes were placed in a light incubator at 28 °C, receiving sterile water every other day. Germination percentages were recorded for seven days after plating.

Effect of bacteria on the growth of wheat in pots

A pot experiment was conducted to investigate the influence of two PGPR strains on wheat growth. Three treatments, including two bacterial interventions and an untreated control, were replicated thrice, with each treatment having four pots (14.5 cm × 8.5 cm). Field soil, sterilized through two autoclaving cycles, served as the potting medium. Bacteria-treated and non-treated seeds were sown, and after 7 days, seedlings were thinned to ten per pot. Rhizobacterial colonization was monitored at 2, 3 and 4 weeks. At 4 weeks, plants were uprooted for assessment of root length, shoot length, fresh root weight and fresh shoot weight. Subsequently, the plants were oven-dried, and their dry weight, as well as nutrient concentrations (N, P and K), were determined using inductively coupled plasma spectrometry and the Kjeldahl method. Moreover, the microbial biomass carbon in soils was estimated through the chloroform fumigation extraction method (Vance et al., 1987).

Effect of PGPR on the growth and yield of wheat with reduced nitrogen fertilization doses

A pot experiment assessed the impact of *S. koreensis* RB11 and *B. amiloliquefaciens* RB12 on wheat growth and yield under reduced nitrogen doses. Ten treatments included various nitrogen levels (100%, 75%, 50% and 25%) with control or bacterized seeds. Each treatment had three replications, with four pots per replication. Each pot (14.5 cm × 8.5 cm) was filled with 3.0 kg autoclaved field soil. For 100% doses, N 0.828 g, P_2O_5 0.392 g, and K_2O 0.209 g were used in each pot (3 kg soil). Nitrogen was applied in three equal splits, one during soil preparation, and others one month and two months after sowing. P and K were added as full basal doses. Twenty seeds were sown in each pot and after germination, 10 seedlings were allowed to grow in a net house at 25±2 °C. Data on the root, shoot, and grain weights were recorded after harvesting at maturity.

Preparation of spore suspension of *B. sorokiniana*

A seven-day-old culture of *B. sorokiniana* on PDA was flooded with 10 ml of sterilized distilled water. Conidia and mycelial mass were collected using a narrow-edged sterilized glass slide and sieved through cheesecloth to remove mycelial mass. The spore suspension was adjusted to 5×10^5 spores ml^{-1}, counted under a microscope, and supplemented with two drops of Tween 20 per 100 ml suspension.

Effect of bacteria on germination of conidia of *B. sorokiniana*

Bacteria were cultured overnight in NP broth at 28 °C, collected, and suspended in sterilized distilled water. The bacterial concentration was adjusted to 10^8 cfu ml^{-1}. A 5 ml^{-1} aliquot, mixed 1:1 with spores, was incubated for 24 hours at 28 °C. Controls lacked bacterial filtrate. Microscopic examination of at least 100 spores per replicate assessed inhibitory effects on germination, germ tube length, and appressorial formation. Percent inhibition was calculated using Equation 1.

$$\% \text{ Inhibition} = \frac{(A-B)}{A} \times 100 \qquad (1)$$

Where, *A* is the number of germinated spores, germ tubes, and appressoria in the control and *B* is the number of germinated spores, germ tubes, and appressoria in the test samples.

Biocontrol efficacy of PGPR on common root rot and spot blotch caused by *B. sorokiniana*

A pot experiment evaluated the effect of two PGPR strains on common root rot and spot blotch of wheat. The four treatments included two bacterial treatments, an untreated control, and a positive control using Protaf 250EC (0.1%). Each treatment had three replications, with four pots (14.5 cm × 8.5 cm) each. Seeds were prepared, and 20 seeds were sown in each pot. After 7 days, 10 seedlings were retained in each pot and grown in a net house at 25±2 °C. The bacterial suspension (10^8 cfu ml^{-1}) was sprayed on seedlings after 2 weeks, while the positive and negative controls were sprayed with Protaf 250EC (0.1%) and water, respectively. Two days later, a spore suspension of *B. sorokiniana* (5×10^5 spores ml^{-1}) was sprayed on wheat plants. After 48 hours in a moist chamber, plants were moved to the growth room. Disease incidence was evaluated on the 10^{th} day after inoculation by counting infected plants. The incidence of each disease was estimated by counting the number of

infected plants showing the disease symptoms over the total number of plants. On the other hand, disease severity of spot blotch was estimated using a 0 to 5 scale (0 = no visible lesion on leaves, 1 = necrotic spot without chlorosis and up to 5% leaf area covered, 2 = necrotic spots with light chlorosis, 3 = necrotic spots with pronounced chlorosis and 21 to 40% leaf area covered, 4 = lesions enlarging and 41 to 60% leaf area covered and 5 = lesions merging and more than 60% leaf area blighted) (Adlakha et al., 1984). The disease severity index was calculated using Equation 2.

$$\text{Severity Index} = \frac{X}{Y \times Z} \quad (2)$$

Where, X = summation of all ratings, Y = total number of ratings, Z = maximum disease grade.

Design of experiments and analysis of data

The experimental design was conducted in a completely randomized manner, comprising three replications for each treatment. The data presented herein are derived from representative experiments that were repeated a minimum of two times, yielding consistent results. Treatment means were differentiated using Fisher's least significant difference test. All statistical analyses were carried out at a significance level of $p < 0.05$, employing XLSTAT Pro statistical analysis software by Addinsoft (New York, USA).

RESULTS AND DISCUSSION

Morphological, biochemical and molecular characterization of the PGPR strains

Two PGPR isolates, RB11 and RB12, were selected for their strong antagonistic activity against fungal pathogens, displayed rod-shaped morphology, non-pigmented appearance, halotolerance (≤ 12% NaCl), and positive reactions towards citrate utilization, catalase, Voges Proskauer, and oxidase (Table 1). RB11 (MH368355.1) exhibited 99.58% similarity to *S. koreensis*, while RB12 (MH368356.1) had 100% sequence similarity to *B. amyloliquefaciens*. Staining tests revealed RB11 as gram-negative and RB12 as gram-positive. Additionally, RB11 showed positive reactions in the KOH solubility test, while RB12 was negative. Previous research also characterized PGPR strains such as *B. amyloliquefaciens* and *Stenotrophomonas* as effective antagonists and highlighted the significance of flagellar motility and citrate utilization in root colonization (Islam et al., 2016). Considering these attributes, RB11 and RB12 emerge as potential new PGPR strains worthy of testing for plant growth promotion and disease control.

Plant growth-promoting and biocontrol traits

Both isolates solubilized phosphate, produced IAA (32.62 to 41.95 µg ml^{-1}) and showed nitrogenase activity (1.78 to 3.99 µmol C_2H_4 mg protein h^{-1}) (Table 2). Additionally, both isolates produced siderophores and HCN, utilized 1-aminocyclopropane-1-carboxylate (ACC) as a nitrogen source, and formed biofilms on glass tube surfaces (OD_{600} 0.21-27). Isolates RB11 and RB12 were tested positive for the chitinase, lipase and protease tests. In the antagonistic assay, both isolates inhibited the mycelial growth of *R. solani*, *B. sorokiniana*, *S. rolfsii* and *S. sclerotiorum*. The findings align with previous studies that

Table 1. Morphological, biochemical and molecular analysis of the PGPR isolates

Characteristic	Test	Rhizobacterial isolates*	
		RB11	RB12
Morphological	Colony color	Creamy white	Creamy white
	Colony margin	Round	Round
	Cell shape	Rod	Rod
	Motility	+	+
	Endospore	-	+
Biochemical	Gram reaction	-	+
	KOH	+	-
	Catalase	+	+
	Citrate	+	+
	Voges Proskauer	+	+
	Oxidase	+	+
	NaCl tolerance	≤ 12%	≤ 12%
Molecular	Similarity-based on 16s rRNA gene sequencing	*S. koreensis* (99.58%)	*B. amyloliquefaciens* (100%)

Note: *+ = positive; − = negative result for the test

Table 2. Plant growth-promoting and biocontrol traits of bacterial isolates

Plant growth-promoting and biocontrol trait	Bacterial isolates	
	S. koreensis RB11	*B. amyloliquefaciens* RB12
Phosphate solubilization	+++	+++
Indole 3-acetic acid (µg m^{-1})	32.62±0.27	41.95±0.49
Acetylene reduction assay (µmol C_2H_4 mg protein h^{-1})	1.78±0.11	3.99±0.21
1-aminocyclopropane-1-carboxylate (ACC) deaminase	++	+++
Biofilm (OD_{600})	0.21±0.01	0.27±0.02
Siderophore	++	++
Hydrogen cyanide (HCN)	+++	++
Chitinase	++	++
Protease	++	++
Lipase	++	+
Cellulase	++	++
*Antagonism in dual culture against (% inhibition of mycelial growth)		
B. sorokiniana BS-1	83.58±0.71	85.73±0.84
R. solani AR-01	76.04±0.67	89.52±0.69
S. rolfsii SR-1	88.04±0.36	77.13±0.42
S. sclerotiorum PSB-1	82.81±0.58	70.76±0.11

Note: +++ = good; ++ = medium; + = slight; − = negative. *The antagonism against fungal pathogens was measured as the percent inhibition of radial growth of the fungal pathogens by PGPR antagonists in a dual plate assay. Values are means±SE (n = 3)

highlighted their diverse plant growth-promoting and antagonistic traits (Islam et al., 2016; Li et al., 2017; Masum et al., 2018; Rahman et al., 2018; Deng et al., 2022). Therefore, the *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 possess a robust combination of plant growth-promoting and antagonistic traits, making them ideal microbial agents.

Effect of seed priming with PGPR on germination

Seeds treated with rhizobacteria exhibited faster and higher germination rates than untreated seeds (Figure 1). The highest germination rate (95.33%) was observed in seeds treated with *B. amyloliquefaciens* RB12, while the control treatment had the lowest germination rate (72.76%). The percent increase in germination for RB11 and RB12-treated seeds over the control was 14.59% and 31.03%, respectively. In previous studies, seed bacterization with *Stenotrophomonas maltophilia* and *B. amyloliquefaciens* improved seed germination of various crops, including wheat (Islam et al., 2016; Rahman et al., 2018; Sultana et al., 2020). This improvement might be attributed to increased IAA production, a crucial

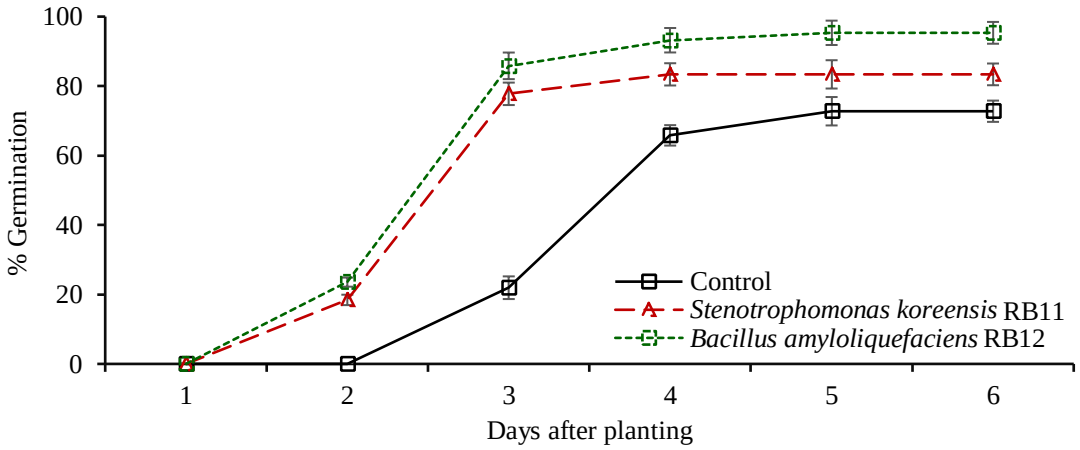

Figure 1. Effect of *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 on seed germination of wheat. Germination of bacterized and non-bacterized wheat seeds was measured daily for up to 6 days

phytohormone known for promoting early seed germination (Prashanth and Mathivanan, 2010). These antagonistic PGPR isolates may also inhibit seed mycoflora, further contributing to enhanced seed germination. Moreover, increased amylase activity in treated seeds breaks down starch molecules into simpler sugars, ultimately improving seed germination (Hossain et al., 2017).

Effect of PGPR on plant growth, root colonization, nutrient contents and biomass carbon

A significant enhancement in wheat growth was evident with PGPR treatment compared to the untreated control (Table 3). Specifically, plants treated with RB11 and RB12 resulted in a 57.34 to 62.39% increase in shoot length over the untreated control, where RB12 displayed the highest improvement. Similarly, significantly higher fresh and dry shoot weights were observed in PGPR-treated plants, with RB12 resulting in an 80.95% and 77.78% increase in fresh and dry shoot weights, respectively. Root lengths, root fresh weights and root dry weights were also significantly increased in plants treated with both *S. koreensis* RB11 and *B. amyloliquefaciens* RB12, with RB12 showing the highest increase (73.46%, 63.64% and 100%). The root colonization assay revealed population densities ranging from 3.5×10^7 to 38.5×10^7 cfu of RB11 and 4.9×10^7 to 79.1×10^7 cfu of RB12 per gram of root tissue across crop stages (Figure 2A). Among the three nutrients, the N and P content in wheat plants was significantly enhanced by PGPR treatment, with RB12 resulting in the highest N (2.49 mg g^{-1}) and P (0.30 mg g^{-1}) level (Figure 2B). The biomass carbon accumulation in the rhizosphere soil exhibited a substantial increase due to rhizobacterial treatments, ranging from 534.55 to 657.37 mg kg^{-1} of soil, with the highest accumulation observed in RB12 colonized soil (Figure 2C). Previous studies have confirmed the growth-promoting effects of *B. amyloliquefaciens* on wheat (Rahman et al., 2018). However, the role of *S. koreensis* in enhancing wheat growth is not well-documented, despite the recognition of *Stenotrophomonas* as a PGPR (Islam et al., 2016; Alexander et al., 2019). The improved plant growth by these bacteria is attributed to key mechanisms such as phosphate solubilization, biological nitrogen fixation, IAA and siderophore production,

Table 3. Effect of *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 on growth of wheat plants

PGPR	Shoot length (cm)	Fresh shoot weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
S. koreensis RB11	25.85±2.02[a]* (57.34%)**	0.34±0.06[b] (61.90%)	0.14±0.01[a] (55.56%)	13.26±1.15[a] (57.11%)	0.16±0.01[a] (45.45%)	0.07±0.01[a] (75.00%)
B. amyloliquefaciens RB12	26.68±1.97[a] (62.39%)	0.38±0.06[a] (80.95%)	0.16±0.02[a] (77.78%)	14.64±1.12[a] (73.46%)	0.18±0.02[a] (63.64%)	0.08±0.01[a] (100.00%)
Control (no PGPR)	16.43±1.32[b]	0.21±0.03[c]	0.09±0.01[b]	8.44±0.98[b]	0.11±0.01[b]	0.04±0.01[b]

Note: *Different letters within the same column indicate a significant difference ($p = 0.05$). Values are means±SE. **Values in the parenthesis indicate a %increase over the control

Table 4. Effect of *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 on growth and yield of wheat under reduced nitrogen fertilization

Treatment	Shoot weight (g)	Root weight (g)	Panicle weight (g)	Grain weight (g)
Full dose N+PK	45.22±3.46[ab]***	11.42±0.98[cd]	56.27±6.36[ab]	45.93±4.12[ab]
2/3N+PK	40.04±2.11[bcd]	9.17±1.00[d]	51.65±5.97[bcd]	39.38±4.79[c]
1/2N+PK	36.68±2.29[cd]	6.85±0.81[f]	48.10±4.34[cd]	36.27±4.09[d]
1/3N+PK	21.54±1.64[e]	6.73±0.74[f]	37.42±4.12[e]	27.25±3.17[f]
2/3N+FD PK+RB11*	47.75±4.78[a]	14.67±1.64[b]	58.42±4.87[a]	46.88±4.48[a]
1/2N+FD PK+RB11	43.25±4.23[b]	12.45±1.45[c]	53.82±6.21[b]	39.61±4.32[c]
1/3N+FD PK+RB11	36.28±3.11[cd]	8.77±0.65[e]	46.24±4.12[d]	31.55±3.57[e]
2/3N+FD PK+RB12**	49.14±4.29[a]	16.78±1.97[a]	57.47±6.32[a]	47.42±5.78[a]
1/2N+FD PK+RB12	41.29±2.42[bc]	13.30±1.24[bc]	52.73±3.45[bc]	40.97±3.24[bc]
1/3N+FD PK+RB12	34.79±3.36[d]	11.72±1.68[cd]	47.48±4.37[d]	33.58±3.41[de]

Note: * Wheat seeds treated with *S. koreensis* RB11. ** Wheat seeds treated with *B. amyloliquefaciens* RB12. *** Values are means±SE (n = 3). Each replication consists of four pots. Different letters within the same column indicate significant differences ($p = 0.05$)

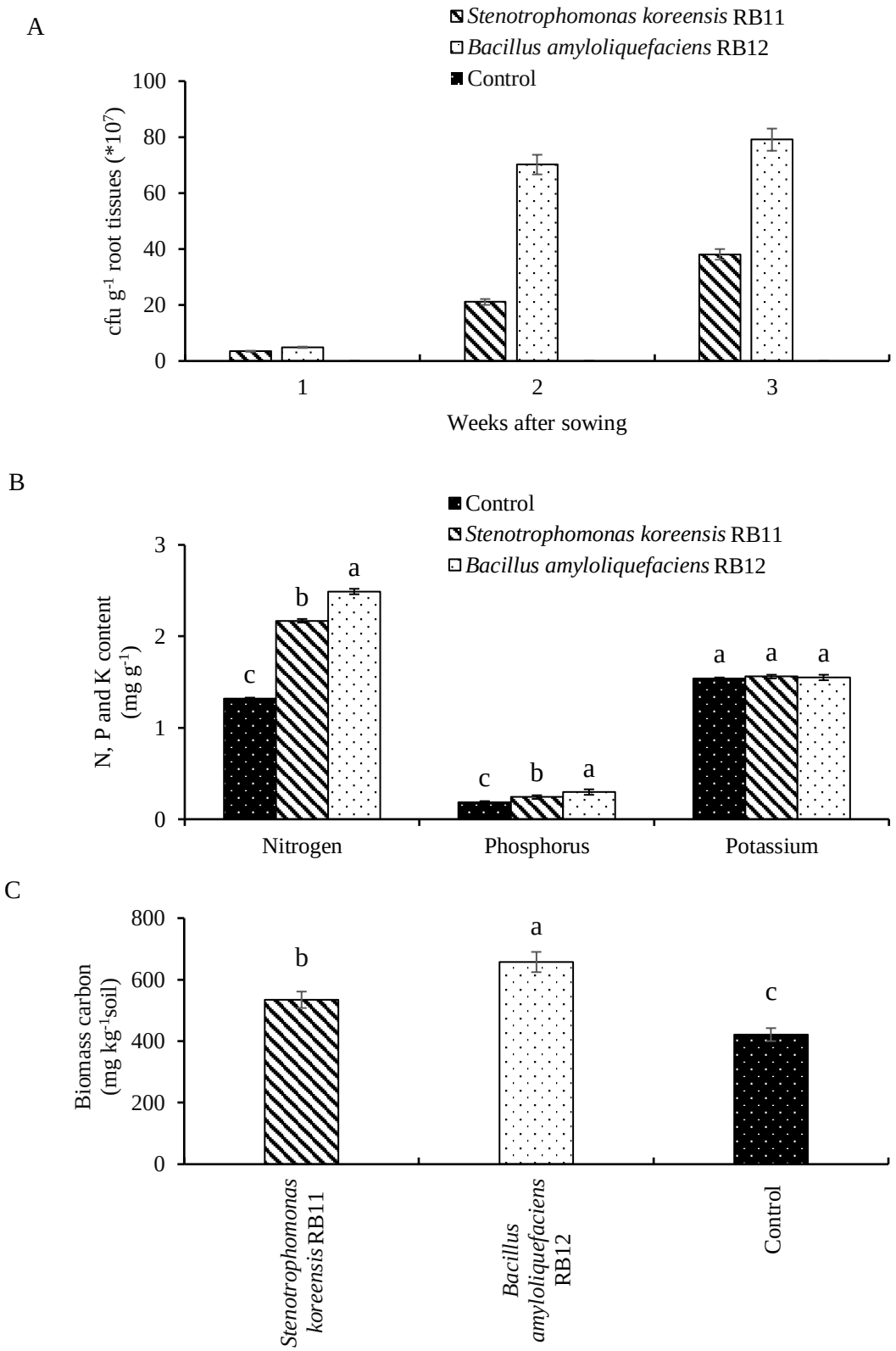

Figure 2. Effect of *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 strains on root colonization (cfu) (A), nutrient contents (N, P and K) (B), and biomass carbon accumulation in rhizosphere soil (C). Error bars are SE from three replicates that received the same treatment. Within each frame, different letters indicate a statistically significant difference between treatments (LSD test, $p \leq 0.05$)

ACC-deaminase activity, biomass carbon accumulation, and efficient root colonization (Prashanth and Mathivanan, 2010; Sultana and Hossain, 2022). These findings suggest that the PGPR strains employ multiple mechanisms to enhance wheat growth.

Effect of reducing fertilizer application

Compared to a 100% N fertilizer application, reducing N doses by 25%, 50% and 75% significantly inhibited plant growth and yield (Table 4). However, supplementing these reduced N doses with rhizobacterial treatment significantly improved plant growth and yield. Consequently, there were no significant differences in shoot weight, root weight, panicle weight, and grain weight between full NPK fertilizer doses and the combination of *B. amyloliquefaciens* RB12 with 75% N fertilizer (+ full PK dose) or 50% N fertilizer (+ full PK dose). Similarly, combining 75%-reduced-nitrogen fertilizer with *S. koreensis* RB11 treatment yielded results comparable to using the full nitrogen dose. Reducing N fertilizer rates by 50% and 25% with *B. amyloliquefaciens* RB12 and *S. koreensis* RB11, respectively, aligns with prior findings. In a greenhouse, tomato transplants with 75% fertilizer and co-inoculated PGPR exhibited significantly greater dry weight than those with full fertilizer without PGPR (Hernandez and Chailloux, 2004). The inclusion of PGPR in soils with a 50% organic and 50% conventional N fertilizer mix resulted in Kikuyu grass yields similar to those achieved with 100% conventional N fertilizer (Paungfoo-Lonhienne et al., 2019).

Inhibition of conidial germination

After a 24-hour incubation, both bacteria significantly reduced *B. sorokiniana* conidial germination compared to the control (Table 5). Conidia treated with *B. amyloliquefaciens* RB12 and *S. koreensis* RB11 showed germination rates between 10.66% and 14.67% compared to 89.33% in controls, resulting in an inhibition range of 88.07 to 83.58%. Both rhizobacteria also inhibited appressoria development and germ tube length, with *B. amyloliquefaciens* RB12 recording the highest inhibition rate of 91.52% and 87.71%, respectively. On the other hand, *S. koreensis* RB11 exhibited inhibition rates of 87.71% for appressoria formation and 83.71% for germ tube length. These findings highlight the potent antifungal activity of both rhizobacteria against *B. sorokiniana*. *B. amyloliquefaciens*, known for its broad antifungal spectrum, effectively inhibits spore germination of *B. sorokiniana* (Yi et al., 2021). Although there are no specific references for *S. koreensis*, a related species, *S. maltophilia*, has been reported to inhibit *B. sorokiniana* spore germination (Zhang and Yuen, 2000). The hydrolytic enzymes, including chitinases, proteases and lipases produced by the bacteria, may induce swelling, vacuolation, and malformation of *B. sorokiniana* conidia and germ tubes, thereby inhibiting their germination (Zhang and Yuen, 2000; Yi et al., 2021).

Suppression of common root rot and spot blotch by PGPR

The occurrence of common root rot and spot blotch was significantly lower in plants treated with rhizobacteria and fungicide compared to the control group (Figure 3). Untreated controls exhibited a common root rot incidence of 27.54% and spot blotch severity index of 3.61 (Figure 3A and 3B). However, plants treated with RB11 showed a common rot incidence of 15.24% and a spot blotch severity index of 2.14, while those treated with RB12 displayed a common root rot severity index of 11.17% and a spot blotch severity index of 1.71. These values were statistically comparable to those observed in plants treated with the fungicide Protaf 250EC (0.1%). Notably, RB11 and RB12 reduced common root rot by 44.66% and 59.44% and spot blotch severity by 40.72% and 52.63%,

Table 5. Co-culture effect of *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 on the spore germination, appressoria formation, and germ tube length of *B. sorokiniana* BS-1

Treatment	Conidial germination (%)	Appressoria formation (%)	Germ tube length (µm)
S. koreensis RB11	14.67±1.41[b]* (83.58)**	9.67±0.75[b] (87.71)	16.05±1.34[b] (83.71)
B. amyloliquefaciens RB12	10.66±0.87[c] (88.07)	6.67±1.04[c] (91.52)	11.60±0.86[c] (88.23)
Control (no biocontrol)	89.33±0.87[a]	78.67±6.24[a]	98.54.24±6.11[a]

Note: *Values are means±SE (n = 3); one replication consists of 100 spores. **Values within parenthesis indicate the % reduction over control

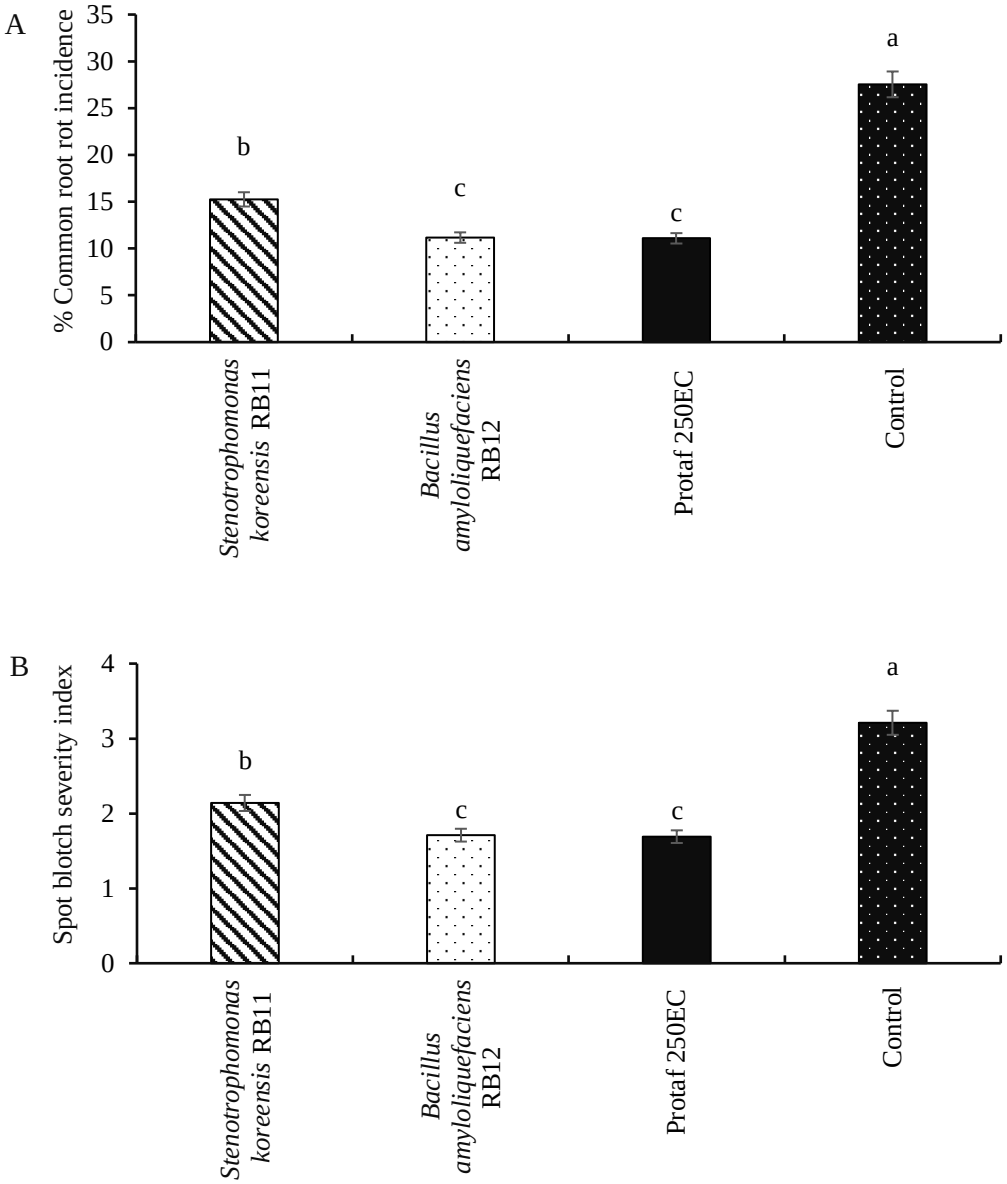

Figure 3. Effect of *S. koreensis* RB11, *B. amyloliquefaciens* RB12, and fungicide Protaf 250EC on common root rot (A) and spot blotch severity index (B) caused by *B. sorokiniana* in wheat. Error bars are SE from three replicates that received the same treatment. Within each frame, different letters indicate a statistically significant difference between treatments (LSD test, $p \leq 0.05$)

respectively. The findings suggest the potential efficacy of *S. koreensis* RB11 and *B. amyloliquefaciens* RB12 in controlling *B. sorokiniana* in wheat. Previous studies have highlighted the biocontrol capabilities of *B. amyloliquefaciens* and *S. maltophilia* against *B. sorokiniana* in Poaceae plants (Zhang and Yuen 2000; Yi et al., 2021). The biocontrol mechanisms by these bacteria seem to involve both direct and indirect pathways. Notably, these bacteria produce HCN, siderophores, and hydrolytic enzymes, which are vital for their antifungal capabilities. These substances may directly inhibit the mycelial growth of the fungal pathogen, disrupt its spore germination, and impede appressoria formation, thus, effectively preventing the successful infection by the pathogen. Moreover, the possibility of triggering induced systemic resistance (ISR) by PGPR against *B. sorokiniana* cannot be ruled out

(Kilic-Ekici and Yuen, 2004; Yi et al., 2021), suggesting the potential involvement of multiple mechanisms.

CONCLUSIONS

This study underscores the significant roles of two PGPR strains, *S. koreensis* RB11 and *B. amyloliquefaciens* RB12, in enhancing plant growth and providing protection against *B. sorokiniana* in wheat. These strains exhibit diverse plant growth-promoting and biocontrol traits, effectively controlling common seedling rot and spot blotch while promoting plant growth and yield under reduced nitrogen fertilizer application. The observed disease protection effect is comparable to that of the fungicide Protaf 250EC (0.1%). These findings highlight the potential for tailored PGPR-based agricultural approaches, offering promising opportunities to reduce chemical inputs for improved crop growth and disease control. Further studies can elucidate the precise mechanisms by which these two bacteria stimulate growth and control *B. sorokiniana* in wheat.

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3eSwIr8M4U&redir_esc=y#v=onepage&q=Application%20and%20mechanisms%20of%20plant%20growth%20promoting%20fungi%20(PGPF)%20for%20phytostimulation&f=false

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