Technology in Agronomy

ARTICLE

Open Access

https://doi.org/10.48130/tia-0025-0005

Technology in Agronomy **2025**, 5: e010

Harnessing bacterial bioagents to control sheath blight of rice

Md Arraf Hasan Reedoy[1], Jannatul Ferdos Shimu[2], Irfan Khan[1], Mangal Shahi[1], Md Hasibul Hasan[1], Sanjida Parven Sarly[1], Masfia Rahman[1], Afrina Rawshan Dipty[1], Uday Rana Singha[3], Shah Tasdika Auyon[4] and Md Rashidul Islam[1*]

[1] *Plant Bacteriology & Biotechnology Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[2] *Department of Agronomy, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[3] *Department of Agricultural Extension, Khamarbari, Dhaka 1215, Bangladesh*
[4] *Department of Environmental Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
* Corresponding author, E-mail: rashidul.islam@bau.edu.bd

Abstract

Sheath blight is caused by the fungal pathogen *Rhizoctonia solani* which causes yield losses of 20% up to 70%. While fungicide application is indeed effective against *R. solani* and can be safe when applied correctly, improper applications can have potentially negative effects on the environment and animals and may lead to fungicide resistance in pathogen subpopulations. The present study aimed to evaluate the efficacy of formulated bacterial bioagents in controlling sheath blight pathogen *R. solani* and sheath blight disease of rice to assess their performance in increasing rice yield. Out of 23 bacterial isolates, four bacterial isolates of different species viz. BDISO45JoyR (*Bacillus subtilis*), BDISO49JoyR (*B. subtilis*), BDISOB219R (*Pseudomonas taiwanensis*), and BDISOB221R (*Pseudomonas* sp.) were found to be the most effective against *R. solani*. These four bacterial isolates were formulated using talcum powder as a carrier material in the laboratory. Under field conditions, the formulated bacterial bioagents significantly reduced the lesion length and tiller infection of sheath blight, with BDISO45JoyR (*B. subtilis*) showing a maximum reduction of 55.25% and 54.80%, respectively, over the untreated control. The minimum reduction of lesion length and tiller infection was 28.30% and 32.17% was recorded in BDISOB219R (*P. taiwanensis*). BDISOB221R (*Pseudomonas* sp.) showed a maximum plant height of 116.5 cm. All the formulated bacterial bioagents showed better performance over control that increased yield. The maximum yield of 5.28 t/ha with a 58.51% increase over control was observed with BDISO45JoyR (*B. subtilis*) treatment compared to the untreated control. Therefore, bacterial bioagents should be considered for use in disease prevention and for the healthy growth of plants.

Citation: Reedoy MAH, Shimu JF, Khan I, Shahi M, Hasan MH, et al. 2025. Harnessing bacterial bioagents to control sheath blight of rice. *Technology in Agronomy* 5: e010 https://doi.org/10.48130/tia-0025-0005

Introduction

Rice (*Oryza sativa* L.) is an extensively cultivated cereal crop that provides essential food support to more than half of the people on Earth[1]. This major cereal crop retains pivotal importance for worldwide food security since it satisfies most individuals who reside beneath the poverty line[2]. In Bangladesh, rice is farmed on around 11.7 million hectares, or 77% of the cultivable area, through three distinct growing seasons called Aus, Aman, and Boro (named after their separate growing times)[3]. Boro is an irrigation-dependent season because of the lack of rainfall at this time, while Aus and Aman are completely tropical monsoon rain-dependent seasons for rice cultivation[4]. Approximately 522 million metric tons of rice are produced globally each year, whereas over 39.1 million metric tons are produced in Bangladesh for 169 million people[5,6]. A staple crop throughout the world, rice provides up to 76% of Southeast Asians' calorie consumption and more than 21% of human caloric needs[7]. The demand for rice has increased in Bangladesh, although the area planted to rice is not growing, the country's growing population requires quick growth in rice production. The low rice yield in Bangladesh is as a result of a number of causes. Among these, the crops' susceptibility to diseases and pests is crucial. Various types of pathogenic microbes are responsible for rice diseases. Five diseases, such as sheath blight, bacterial blight, blast, tungro, and ufra have been identified as the most serious among them due to their widespread incidence and substantial damage potential[8]. Over 50% of the world's rice output is affected by sheath blight, a serious disease caused by the fungus *R. solani*[3], which can spread across large areas and lead to yield losses of up to 70%[9]. Sheath blight affects the entire life cycle of rice, from seedling to heading stage, causing wilting of leaves and sheaths as well as a reduction in seed laying rates[9]. The pathogen hampers rice growth and yield by causing lodging, low yield, and poor grain quality, while also infecting the soil with pathogens and disrupting its biochemical compositions. Large areas may be affected by the disease, which can result in a yield loss of up to 70%[3]. The disease primarily affects the lower leaf sheaths at the maximal tillering or early internode elongation stages of plant growth[10]. The infection spreads swiftly through hyphal runners, which rise from the roots to higher plant parts like leaf blades and other plants, especially in the early phases of grain filling and heading growth[11]. Transplanted aus rice suffers from the worst disease intensity, followed by aman and boro rice[12].

Recovering from sheath blight diseases requires more initiatives than just using conventional rice cultivars or synthetic fungicides to target sustainable rice production[13]. The main strategy for managing rice sheath blight is chemical control, although there are only a few fungicides available for this disease[14]. Currently, the primary methods for treating and preventing the disease are chemical fungicides and cultivation techniques[15,16]. In the field, a variety of fungicides were effective in managing sheath blight disease, including benomyl, captafol, carbendazim, carboxy, chloroneb, edifenphos, mancozeb, thiophanate, and zineb[17,18]. However, there is a chance that fungicide-resistant organisms will arise as a result of ongoing usage of these pesticides, making future disease control more difficult[19], and results in long-term damage to human health and the environment[20]. Fungicide resistance has increased as a result of the frequent and excessive use of these products. Serious public health concerns, particularly in developing nations, are the acute

and long-term health impacts of dietary exposure to agricultural pesticides. Chemical pesticides have the potential to be mutagenic, cytotoxic, and carcinogenic to human health[21]. On the other hand, most of the promising cultivars are not able to survive the pathogen, and there are no known resistant cultivars against it[11]. Fungicides that are resistant to diseases like blast and powdery mildew are found in Mediterranean countries. To control conditions, various methods such as biocontrol and integrated pest and disease management techniques are taken into consideration[22]. Utilizing biological organisms such as *Bacillus* sp. and *Pseudomonas* sp. to reduce crop disease rates is a tried-and-true method when taking environmental sustainability into account[23]. Nowadays, most individuals have adopted biological ways for growing plants because they are aware of the risks associated with fungicides. In India, *Phythium aphanidermatum*-induced damping off of mustard is well managed by *Trichoderma viridae* mutants[24]. They interfere with certain microbes' metabolic processes, which eventually kill the organisms that are infected. Furthermore, bio-control agents work well for diseases caused by soil such *Rhizoctonia* sp., *Pythium* sp., and *Fusarium* sp.[25,26].

Bacillus subtilis, a gram-positive bacterium that belongs to the order Bacillales of the family Bacillaceae, is one of the main biocontrol agents used today. It shows promise in managing microbial illnesses that affect both plants and soil. Up to 90% of sheath blight disease can be controlled by Bacillus strains that block *R. solani* growth[27,28]. Similarly, *Pseudomonas fluorescens*, a gram-negative bacterium genus belonging to the Pseudomonadaceae family, is also employed extensively as a biocontrol agent to prevent foliar infections brought on by soil-borne infectious illnesses. It is one of the most promising rhizosphere bacteria since it both inhibits disease and encourages plant growth[29]. Additionally, it can avoid plant diseases like soft rot, powdery mildew, and sheath blight. The genus Pseudomonas exhibits resistance to microbial attack on plants and soil because of a variety of processes, including the generation of enzymes, siderophores, volatile organic compounds, biofilms, and other efficient actions[30]. In Bangladesh, a significant amount of research has been conducted on the formulation and application of *Pseudomonas* sp. and *B. subtilis*. Its application against the rice sheath blight pathogen *R. solani*, however, is insufficiently understood.

Antagonistic bacteria have become a viable biocontrol weapon against sheath blight because of their capacity to suppress *R. solani* growth and activity. To assess the efficacy of bacterial bioagents in practical settings, field tests are necessary. Therefore, the purpose of this study was to find possible bacterial bioagents that could inhibit *R. solani*, such as Bacillus subtilis and Pseudomonas sp. The study specifically aimed to determine their efficacy in lowering rice sheath blight in field settings and increasing rice yield while also evaluating their capacity to inhibit the radial mycelial proliferation of *R. solani*.

Materials and methods

The experiment was carried out in Plant Bacteriology and Biotechnology Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and in a farmer's field, Sutiakhali, Mymensingh Sadar, Mymensingh, Bangladesh. Experiments were conducted during the period of October, 2022–September, 2023.

Sources of *Rhizoctonia solani* and its isolation

Infected rice plants were collected from a farmer's field in Sutiakhali, Mymensingh Sadar, Mymensingh, Bangladesh, and taken to the Plant Bacteriology and Biotechnology Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. The leaf sheaths were then removed from the infected plants and cut into small pieces, about 1 cm long, containing the growing lesion. After that, the pieces were surface sterilized with 1% Clorox (1g NaOCl per 100 mL) for 3 min and washed three times with sterile water. They were then blot-dried and placed on a Potato Dextrose Agar (PDA) plate, which was incubated at 28 °C for 3 d. After incubation, actively growing mycelium were transferred to a fresh plate to obtain a pure culture, and finally, a slide was prepared and examined under a microscope.

Identification of fungal isolates by sequencing of the ITS region

One hundred ml potato dextrose broth was prepared and the sample was cultured for 7 d. After that, the mycelia were harvested and blot dried with sterile Whatman filter paper. Then 400 mg of mycelium was used for DNA extraction using the Wizard DNA extraction kit. The genomic DNA of fungal isolates was extracted using Wizard® genomic DNA purification kit (Promega, Madison, WI, USA). Extraction of genomic DNA from fungus was quantified by using a UV spectrophotometer absorbance at 260 nm with a model T-80 UV/VIS and stored at −20 °C. Then DNA concentration was adjusted to 100 ng/μL and verified by comparing it with a 100 bp DNA ladder (Invitrogen, USA) on 1.5% agarose gels.

DNA extraction procedure

Leaf tissues were initially frozen with liquid nitrogen and ground into a fine powder using a mortar and pestle. A 40 mg sample of the powdered tissue was transferred to a 1.5 ml microcentrifuge tube, followed by the addition of 600 μL of Nuclei Lysis Solution. The mixture was vortexed briefly and incubated at 65 °C for 15 min. RNase Solution (3 μL) was then added to the lysate, mixed by inversion, and incubated at 37 °C for 15 min. After cooling, 200 μL of Protein Precipitation Solution was added, and the sample was vortexed before being centrifuged at 13,000–16,000 × g for 3 min. The supernatant was carefully transferred to a new tube containing 600 μL of isopropanol, and the DNA was precipitated by gentle mixing. The sample was centrifuged again, and the supernatant was discarded. The DNA pellet was washed with 70% ethanol and centrifuged once more. After carefully removing the ethanol, the pellet was air-dried for 5 min and rehydrated with 25 μL of DNA Rehydration Solution at 65 °C for 1 h. The DNA solution was stored at 2–8 °C for further use.

Primers and PCR conditions

After isolation, the fungal samples were identified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'), and ITS4 (5'-TCCTC CGCTTATTGATATGC-3') specific to Internal Transcribed Spacer (ITS) regions of rDNA from the fungal isolate[31]. The PCR reaction was carried out by Hotstart Master mix (Promega, USA) with genomic DNA as a template. PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 35 cycles at denaturation at 95 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. The final extension was at 72 °C for 6 min. BLAST program was used to determine the species to identify the fungal isolate. PCR products were visualized in 1.5% agarose gel containing ethidium bromide using a Gel Documentation System after separating the PCR products in the agarose gel for 50 min at 80 v.

Bacterial isolates

Twenty-three bacterial isolates were used in this study that were identified previously in the Plant Bacteriology and Biotechnology Laboratory against the bacterial blight pathogen of rice[32]. In this study, these bacterial isolates were used to evaluate their efficacy in

growth inhibition against the sheath blight pathogen *R. solani* (Supplementary Table S1).

Assessment of *in vitro* mycelial growth inhibition of *R. solani* through dual culture assay

The experiment was conducted in a Completely Randomized Design (CRD) with three replications. To assess the *in vitro* growth inhibition of *R. solani* by the bacterial isolates, Nutrient agar-Potato dextrose agar (NA-PDA) (1:1) medium was used as described in a previous study[33]. It was autoclaved at 121 °C, 15 PSI for 15 min and the medium was left to cool for 30 min before plating. The *in vitro* culture (double line method) method was used to test 23 bacterial isolates for their ability to suppress the growth of *R. solani*. A 6 mm mycelial block was taken from 4-day-old *R. solani*, and placed at the center of the NA-PDA medium. Single bacterial colonies were streaked in a straight line (40 mm in length) on both sides of the mycelial plug, at a distance of 25 mm from the fungal mycelial plug, in NA-PDA media. The control plate had no bacterial lines and only had a mycelial plug at the center of the Petri dish. These plates were incubated for 5 d at 28 °C. On the 5th d, mycelial growth data (mm) were recorded using a ruler. The radial mycelial growth of *R. solani* was measured after the incubation period, and the % inhibition was calculated using the following formula[10]:

$$\% \text{ Inhibition} = (C - T)/T \times 100$$

where, C = Growth of *R. solani* in control, and T = Growth of *R. solani* in treatment.

Field experiment and design

Field experiment's were conducted in the same farmer's field of Sutiakhali, Sadar Mymensingh, Bangladesh from December 2022–May 2023. The experiments were carried out in a farmer's field using a Randomized Complete Block Design (RCBD) design with four replications and a plot size of 1 m × 1 m each.

Treatments of the experiment

The following bacterial bioagents were used for field experiment based on their performance under *in vitro* growth conditions against *R. solani* - BDISO45JoyR (*B. subtilis*); BDISO49JoyR (*B. subtilis*); BDISOB219R (*Pseudomonas taiwanensis*); BDISOB221R (*Pseudomonas* sp.). Here, BD: Bangladesh, ISO: Isolate, B: Bacteria, 'Joy' represents the first three letters of the Joypurhat region, and R: Rhizosphere.

In this study, selected treatments were: (1) T_0 = Untreated control; (2) T_1 = Positive control (where the plants are sprayed with Amistar Top 325 SC @ 1 mL/L of water with the active ingredient azoxystrobin and difenoconazole); (3) T_2 = [BDISO45JoyR (*B. subtilis*)], T_3= [BDISO49JoyR (*B. subtilis*)]; (4) T_4= [BDISOB219R (*P. taiwanensis*)]; (5) T_5= [BDISOB221R (*Pseudomonas* sp.)]

Amistar Top 325 a fungicide launched by Syngenta, Bangladesh, and is the most widely used fungicide for field farming to fight sheath blight. The treatments from T_0 to T_5 were sprayed at the active tillering stage at 60 DAT. Bacterial formulation was hand sprinkled on the plant surface by hand @ 6.4 g per plot. One mL of chemical solution was suspended in 1 L of water and sprayed by knapsack sprayer. Every time hands were washed and sprinkled separately for each isolate.

Field condition and rice variety

For this investigation, the BRRI dhan58 rice variety was chosen. Although BRRI already declared BRRI dhan58 to be resistant to sheath blight, in certain developing regions of the nation, this variety is now exhibiting sheath blight susceptibility. BRRI dhan58 was chosen for this study as a result.

The experimental plots were fertilized with urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum, and zinc sulphate at a rate of 225, 52, 60, 30, 5.5 kg/ha, respectively. The entire amounts of triple super phosphate, muriate of potash, gypsum, and zinc sulphate were applied at the time of final land preparation. Urea was applied in three equal installments at 15, 30, and 45 d after transplanting (DAT). After soaking overnight, the seeds were planted on the farmers land, and 30 d old seedlings were collected and transplanted in well-prepared puddled fields at the rate of four seedlings per hill, maintaining 20 cm × 15 cm spacing. The experimental plots were irrigated as and when it was necessary. During the whole growth period, two hand weedings were done, the first weeding was done at 25 d after transplanting followed by a second weeding at 45 DAT.

Preparation and preservation of talc-based formulation for selected bacterial bioagents

Five g carboxy methyl cellulose (CMC) and 7.5 g calcium carbonate was added to 500 g talc powder. This mixture was autoclaved for two consecutive days at 121 °C under 15 PSI pressure for 30 min each. Calcium carbonate was used to adjust the pH to 7.0, CMC to enhance adhesion, glycerol to stabilize bacteria during freezing, and talc powder to maintain bacterial viability, ensuring formulation stability.

The pure cultures of bacterial bioagents were cultured on Lauria Bertani (LB) agar medium for 24 h[10], and the bioagent was grown in LB broth for about 6 h by taking a loopful of bacteria from the LB agar plate. After that, the liquid culture was centrifuged and the pellet resuspended in previously prepared 200 mL peptone broth aimed to fortify with the bactopeptone. This culture broth was then grown for another 2 h with shaking. After that 5 mL of sterile 100% glycerol was added to this 200 mL culture. Then the cultures of the bacterial bioagents (200 mL water fortified with 5 g peptone and 5 mL glycerol 1% of the carrier materials) were added to 500 g sterile talc powder in the tray and mixed well. The formulations were then dried over-night. After that the formulations were powdered manually, and the formulated bacterial antagonists were packed in plastic bags. The shelf life or viability of the formulated bacterial bioagents were also checked (Supplementary Fig. S1).

Artificial inoculation of *R. solani* in rice plants of the experimental plots

Maize sand meal (MSM) media was prepared and the bags filled with MSM media were sterilized at 121 °C, 15 PSI for 20 min in an autoclave, twice at 24-h intervals[34]. Briefly, the *R. solani* culture was taken from the Plant Bacteriology and Biotechnology Laboratory, BAU, which was identified by sequencing of the ITS region in this study. Three 9 mm mycelial discs were taken from 4-d-old actively growing *R. solani* cultures from PDA plates. These blocks were cut from the periphery of the plates with the help of a cork-borer. Three mycelial discs were inoculated into the MSM media in a laminar air flow cabinet. Then the bag was tied tightly after releasing air from the bag. The inoculated bag was kept at room temperature until white colored mycelial were completely covering the media. Any contamination observed in the bags was immediately discarded.

Inoculation of *R. solani* was done at active tillering stage, 60 DAT on the same day as bacterial bioagent application to assess the efficacy of the bacterial bioagents in inhibiting the radial mycelial growth of *R. solani*. Fifteen-day-old MSM media with mycelium were mixed well by shaking and breaking sand clogs. About 1.04 g per plot of the media with sufficient inoculum of the pathogen were uniformly distributed by hand near the waterline in experimental plots. Regular observations on the development of initial symptoms as elliptical to oval greenish-grey spots in sheaths near the waterline were made at 24 h intervals.

Assessment of sheath blight disease in the experimental plots

Sheath blight disease was assessed on the following parameters: (1) Lesion length (cm) at 70 and 81 d after transplanting (DAT). (2) Number of infected tillers 70 and 81 d after transplanting (DAT). The % of disease incidence was calculated using the following formula[35]:

$$\text{Sheath blight incidence (\%)} = \frac{\text{No. of sheath blight infected plants}}{\text{Total no. of plants}} \times 100$$

Briefly, 1 = 0% blight (no disease observed), 2 = 0.1% blight (a few scattered plants blighted; no more than 1 or 2 spots in a 12-yard radius), 3 = 1% blight (up to 10 spots per plant; or general light infection), 4 = 5% blight (about 50 spots per plant; up to 1 in 10 leaflets infected), 5 = 25% blight (nearly every leaflet infected, but plants retain normal form; plants may smell of blight; field looks green although every plant is affected), 6 = 50% blight (every plant affected and about 50% of leaf area destroyed), 7 = 75% blight (about 75% of leaf area destroyed; field appears neither predominantly brown or green), 8 = 95% blight (only a few leaves on plants, but stems green), and 9 = 100% blight (all leaves dead, stems dead, or dying).

Harvesting and data collection on growth and yield parameters

The crops were harvested at the full maturity stage. The maturity of crops was determined when 90% of the grains became golden yellow in color. Then the harvested crops of each plot were bundled separately, properly tagged, and brought to the floor. After harvesting, data were collected on the following parameters: (1) Plant height (cm); (2) Length of panicle (cm); (3) Number of grains per panicle; (4) Number of chuffy grains per panicle; (5) Weight of 1,000 grains; (6) Yield (t/ha).

Data were collected on lesion length (cm) which was measured using a 15 cm ruler at 10 and 21 d after inoculation for field experiments using the following formula:

$$\text{\% Reduction of lesion length} = \frac{\text{Lesion length in control plant} - \text{Lesion length in treated plant}}{\text{Lesion length in control plant}} \times 100$$

Yield data was recorded at the time of harvest and fresh weight was converted at 14% moisture content using the following formula:

$$\text{Dry weight} = \frac{\text{Fresh weight } (100 - \text{Moisture content at harvest } (30\%))}{100 - \text{Desired moisture content } (14\%)}$$

Data analysis

The data recorded on different parameters were tabulated in Microsoft Excel 2010 and documented under the RCBD design. Analysis of Variance (ANOVA) was performed using the 'agricolae' package in R-Studio (R version 3.5.3). To compare means, Duncan's Multiple Range Test (DMRT) was applied to the analyzed data at a significance level of 5%.

Results

Molecular identification of fungus

Fungal isolate was identified by sequencing of ITS (internal transcribed spacer) region using ITS-1 and ITS-4 primers, and PCR products were then sequenced. Analysis of sequencing data of amplified ITS region using Basic Local Alignment Search Tool (BLAST) program revealed that the selected *R. solani* H230214 showed the highest homology with *R. solani* MT158437 (India) and with *R. solani* KX674518 (Malaysia), and confirmed that the fungal species was *R. solani*. Nucleotide sequence alignment by Mega-11 software showed that the nucleotide sequence of the ITS region of *R. solani* H230214 showed 96.58% homology with *R. solani* MT158437 with the strain present in the NCBI (National Center for Biotechnology Information). The phylogenetic relationships showed that *R. solani* was distributed in four clusters. The strain *R. solani* H230214 belonged to cluster 1 which consists of *R. solani* (MT158437) in India, and *R. solani* KX674518 in Malaysia (Fig. 1)[36].

In vitro growth inhibition of *R. solani* in dual culture assay

Of the 23 bacterial bioagents of different genera *viz* BDISO45JoyR (*B. subtilis* strain SA9), BDISO49JoyR (*B. subtilis* strain K24), BDISOB219R (*P. taiwanensis* strain GGRJ11), and BDISOB221R (*Pseudomonas sp*. strain M2.2.1) were found more effective in controlling radial mycelial growth of *R. solani* (Fig. 2). The isolate BDISO45JoyR (*B. subtilis* strain SA9) showed radial mycelial growth (10.17 cm) which was 76.89% reduction over the control treatment of the dual line method. For the isolate BDISO49JoyR (*B. subtilis* strain K24), BDISOB219R (*P. taiwanensis* strain GGRJ11) and BDISOB221R (*Pseudomonas* sp. strain M2.2.1) the radial mycelial growth was 16.33, 22.67, and 21.42 cm respectively (Table 1).

Effect of formulated bacterial bioagents in reducing sheath blight infection of rice under field conditions

Although this study offers insightful information about the efficacy of bioagents, it is crucial to acknowledge the limitations resulting from the trial's one-year, single-location design. This scope might not adequately represent the bioagents' wider effects under various circumstances. To further understand the impacts of the bioagents, more research is also required to examine how lesions arise and spread in tillers. Examining the incidence and length of lesions in tillers may provide crucial information that makes the findings more relevant.

Percent lesion length reduction of sheath blight infection

Plants treated with T_2 exhibited a 55.25% reduction in lesion length at 70 d after treatment (DAT) in comparison to the control. The reductions for treatments T_5, T_3, and T_4 were 46.96%, 39.78%, and 35.91%, respectively (Table 2). Plants treated with T_2 showed a 48.30% reduction in lesion length by 81 DAT, whereas plants treated with T_5, T_3, and T_4 showed reductions of 38.87%, 31.32%, and 28.30%, respectively (Table 2). Under field conditions, T_2 achieved the highest lesion reduction, but T_1, the positive control, outperformed the other treatments overall (Table 2). At 70 DAT, treatments were most effective, and this is probably the economic threshold when the pathogen was most dramatically impacted by treatment. After this, the treatments' efficacy began to decline as the plants attained their ideal levels of growth and development.

Percent tiller infection and reduction of the infection of sheath blight of rice

At 70 d after treatment (DAT), plants treated with T_2 had the lowest percentage of tiller infection (45.20%), whereas T_5, T_3, and T_4 had 54.81%, 62.05%, and 67.83% infection, respectively, compared to the control (Table 2). By 81 DAT, plants treated with T_2 had the lowest tiller infection rate (55.72%) compared to the control (Table 2). T_5, T_3, and T_4 had tiller infection rates of 67.69%, 75.11%, and 78.05%, respectively, while the positive control (T_1) worked better than all other treatments (Table 2).

To increase readability and clarity, graphical representations of reduced lesion lengths and tiller infection percentages were incorporated into the tables (Fig. 3). Although these measures transmit comparable information, displaying them from different angles would provide a full picture of the bioagents' performance.

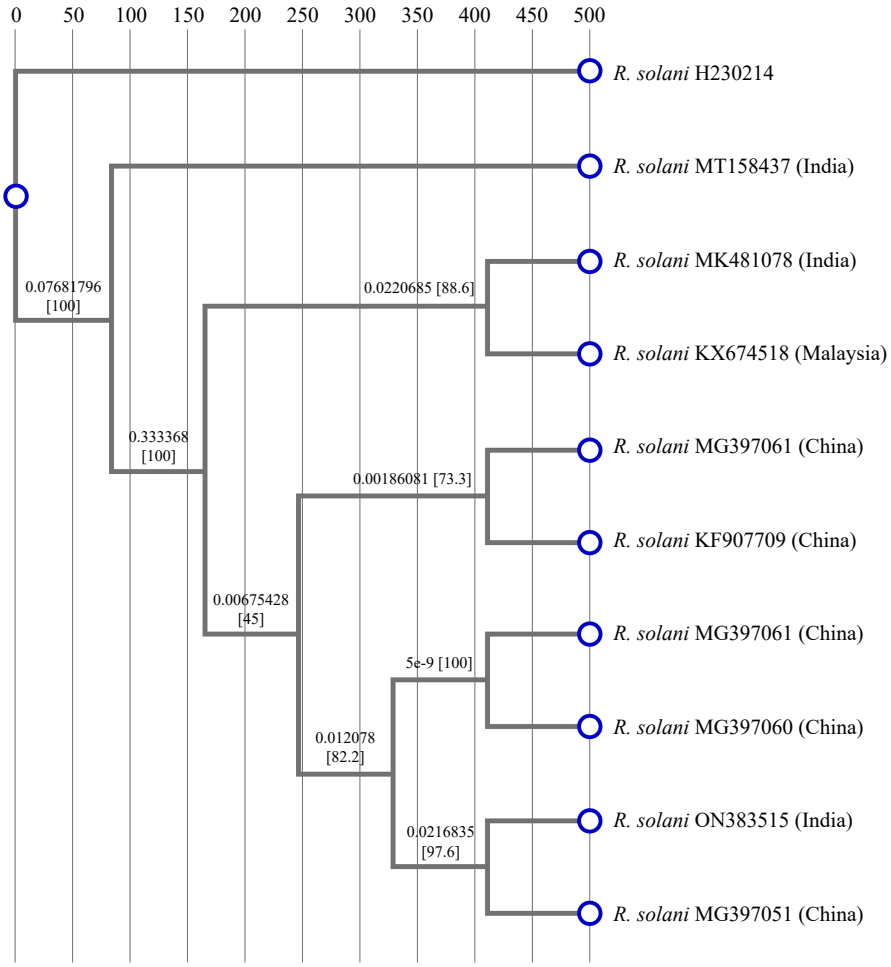

Fig. 1 Phylogenetic relationship of *R. solani* H230214 deposited in NCBI. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown next to the branches.

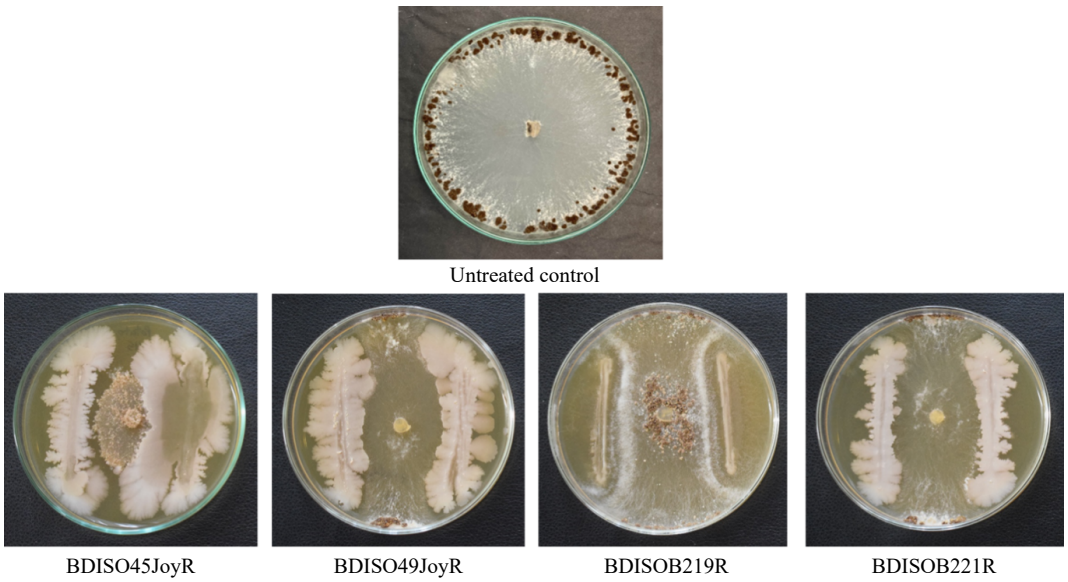

Fig. 2 *In vitro* growth inhibition of *R. solani* by bacterial bioagents. BDISO45JoyR = *B. subtilis*, BDISO49JoyR = *B. subtilis*, BDISOB219R = *P. taiwanensis,* and BDISOB221R = *Pseudomonas* sp.

At 70 DAT, among the bacterial bioagents used in this study, plants treated with T_2 showed a 54.80% reduction in tiller infection (Fig. 4). The treatments with T_5, T_3, and T_4 resulted in 37.97%, 32.17%, and 45.20% reductions in tiller infection over the control, respectively (Fig. 4). By 81 DAT, the plants treated with T_2 showed a 44.27% reduction in tiller infection, while T_5, T_3, and T_4 showed reductions of 24.90%, 21.95%, and 32.31%, respectively (Fig. 4). The positive control, T1, performed better than all other treatments

Table 1. The effect of different bacterial bioagents on inhibiting the radial mycelial growth of *R. solani*.

Isolate ID	Name of the bacteria	Mean radial mycelial growth	% Inhibition of radial mycelial growth
BDISO02PanR	*Pseudomonas fluorescens*	28.5 ± 0.14b	35.23 ± 0.3f
BDISO04PanR	*Pseudomonas putida*	44a	0.00g
BDISO38ThaP	*Pseudomonas fluorescens*	27.75 ± 0.75bc	36.93 ± 1.7ef
BDISO56BogP	*Pseudomonas fluorescens*	26.58 ± 0.61bc	39.58 ± 1.3ef
BDISO64RanP	*Pseudomonas putida*	26.92 ± 0.21bc	38.83 ± 0.46ef
BDIS036ThaR	*Bacillus subtilis*	44a	0.00g
BDISO39ThaR	*Bacillus subtilis*	44a	0g
BDISO45JoyR	*Bacillus subtilis*	10.17 ± 0.95g	76.89 ± 2.1a
BDISO49JoyR	*Bacillus subtilis*	16.33 ± 0.55f	62.84 ± 1.2b
BDISO61JamR	*Bacillus subtilis*	25 ± 1.08cd	43.18 ± 2.4de
BDISOBO4P	*Pseudomonas putida*	44a	0.00g
BDISOBO5P	*Pseudomonas putida*	26 ± 0.14bc	40.91 ± 0.31ef
BDISOB219R	*Pseudomonas taiwanensis*	22.67 ± 0.55de	48.48 ± 1.23cd
BDISOB221R	*Pseudomonas* sp.	21.42 ± 0.34e	51.33 ± 0.77c
BDISOB275R	*Pseudomonas putida*	44a	0.00g
BDISOB283R	*Pseudomonas fluorescens*	44a	0.00g
BDISOB306R	*Pseudomonas putida*	28.42 ± 0.07b	35.42 ± 0.15f
BDISO01R	*Bacillus amyloliquefaciens*	44a	0.00g
BDISO04P	*Pseudomonas putida*	26.17 ± 0.14bc	40.53 ± 0.31ef
BDISO45P	*Bacillus paramycoides*	28.42 ± 0.07b	35.42 ± 0.16f
BDISO154P	*Pseudomonas taiwanensis*	44a	0.00g
BDISO356P	*Pseudomonas hibiscicola*	27.58 ± 0.61bc	37.31 ± 1.39ef
BDISOB92R	*Pseudomonas fluorescens*	44a	0g
Level of significance			0.05
CV (%)			2.78
LSD			6.36

Mean values ± standard error. Values with similar letters are statistically similar. BD: Bangladesh, ISO: Isolate, B: Bacteria, First three letters of Location, R: Rhizosphere, and P: Phylloplane.

(Fig. 4). Compared to 81 DAT, the number of tiller infections was much lower at 70 DAT. This suggests that the bioagent treatments were effective in reducing the intensity of the pathogen early on. The bioagents largely controlled the pathogen, leading to a significant reduction in infection levels by 70 DAT.

Effect of formulated bacterial bioagents on growth and yield parameter of rice under field conditions

Among the bacterial bioagents used in this study, plants treated with T_5 exhibited the maximum plant height (116.5 cm), while the lowest plant height was recorded in untreated plants T_0 (Control). In terms of panicle length, plants treated with T_2 showed the maximum length (22.93 cm), with the lowest panicle length observed in untreated plants T_0 (Control). The highest number of grains per panicle (234.75) was found in plants treated with T_2, while untreated plants T_0 (Control) had the lowest number. Regarding chuffy grains per panicle, plants treated with T_2 showed the lowest number (11.5),

Table 2. Potential of different formulated bacterial bioagents in reducing percent lesion length and tiller infection of rice sheath blight under field conditions.

Treatment	Percent lesion length reduction		Percent tiller infection	
	70 DAT	81 DAT	70 DAT	81 DAT
T_0	0e	0e	100a	100a
T_1	78.17 ± 2.04a	72.45 ± 0.72a	19.31 ± 1.73e	26.08 ± 2.13e
T_2	55.25 ± 1.06b	48.30 ± 1.29b	45.20 ± 1.54d	55.73 ± 1.05d
T_3	39.78 ± 3.42cd	31.32 ± 0.98d	62.05 ± 1.74bc	75.11 ± 1.86b
T_4	35.91 ± 0.90d	28.30 ± 0.98d	67.83 ± 0.79b	78.05 ± 1.13b
T_5	46.96 ± 0.91c	38.87 ± 1.00c	54.81 ± 1.64c	67.69 ± 1.55c
Level of significance	0.05	0.05	0.05	0.05
LSD	4.66	3.88	4.77	5.7
CV (%)	8.88	5.24	5.46	7.68

Mean values ± standard error. Values with similar letters are statistically similar. T_0 = Control, T1 = Positive control, T_2 = BDISO45JoyR (*B. subtilis*), T_3 = BDISO49JoyR (*B. subtilis*), T_4 = BDISOB219R (*P. taiwanensis*) and T_5 = BDISOB221R (*Pseudomonas* sp.).

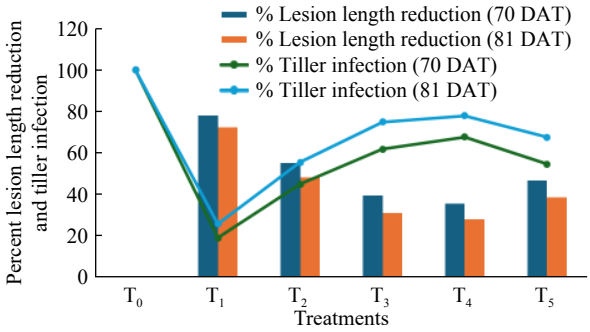

Fig. 3 Effect of formulated bacterial bioagents in percent lesion length reduction and tiller infection of rice sheath blight caused by *R. solani* under field conditions at 70 and 81 DAT. T_0 = Control, T_1 = Positive control, T_2 = BDISO45JoyR (*B. subtilis*), T_3 = BDISO49JoyR (*B. subtilis*), T_4 = BDISOB219R (*P. taiwanensis*) and T_5 = BDISOB221R (*Pseudomonas* sp.).

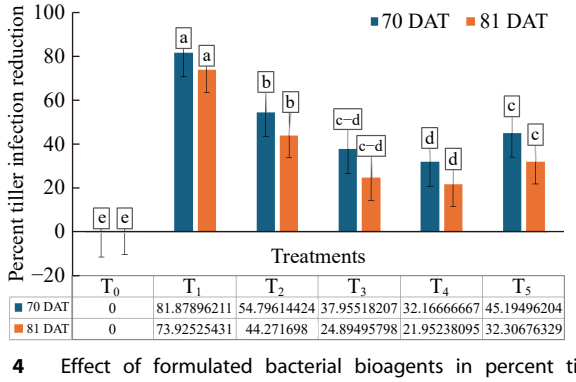

Fig. 4 Effect of formulated bacterial bioagents in percent tiller infection reduction of rice sheath blight caused by *R. solani* under field conditions at 70 and 81 DAT. T_0 = Control, T_1 = Positive control, T_2 = BDISO45JoyR (*B. subtilis*), T_3 = BDISO49JoyR (*B. subtilis*), T_4 = BDISOB219R (*P. taiwanensis*), and T_5 = BDISOB221R (*Pseudomonas* sp.)

while untreated plants T_0 (Control) had the highest. Additionally, plants treated with T_2 had a maximum weight of 1,000 grains (22.44 g), with the minimum recorded in untreated plants T_0 (Control). However, it is important to note that the weight of 1,000 grains is primarily influenced by the genetic material of the rice type, so there was no significant variation in this characteristic. Overall, the positive control T_1 outperformed other treatments (Table 3).

Yield (ton per hectare) and yield increase in percentage

Among the bacterial bioagents used in this study, the plants treated with T_2 showed the maximum yield (5.31 t/ha), followed by T_5 (4.67 t/ha), T_3 (4.59 t/ha), and T_4 (4.67 t/ha). The lowest yield was recorded in untreated plants T0 (Control). Similarly, the plants treated with T_2 showed the maximum yield percentage (59.31%) and the lowest yield percentage was recorded in untreated plants T_0 (Control). However, the positive control T_1 showed better yield and yield increase percentage compared to other treatments (Table 3).

Discussion

Biological control offers a viable alternative to the chemical management strategy for disease with little or no genetic resistance in host plants[35]. However, identifying possible antagonist strains that can successfully suppress the pathogen in a variety of environmental and soil conditions, thrive in the introduced target areas, and enhance crop growth and yield, are the primary requirements for the success of a biocontrol strategy[37]. In our study, the identification of *R. solani* was confirmed by sequencing the ITS region using ITS-1 and ITS-4 primers[38–39]. Among 23 bacterial bioagents, the results in dual culture assay revealed that the percent radial mycelial growth inhibition of *R. solani* exhibited by these bacterial bioagents ranged from 35.23% to 76.89% on the 5th day in the double line method (Table 1). Notably, four bacterial bioagents of different species viz. BDISO45JoyR (*B. subtilis*), BDISO49JoyR (*B. subtilis*), BDISOB219R (*P. taiwanensis*), and BDISOB221R (*Pseudomonas* sp.) were found to be the most effective against *R. solani*, exhibiting 76.89%, 62.84%, 48.48%, and 51.33% inhibition of radial mycelial growth, respectively. In particular, T_5 [BDISOB221R (*Pseudomonas* sp. strain M2.2.1)] has shown 51.33% radial mycelial growth inhibition against *R. solani in vitro*. It is similar to the findings and results which found that 56% reduction in *P. fluorescens* inhibits the mycelial growth and works as an antisporulant to fungal spore development which consequently reduces the disease severity[40]. This kind of bacterial behavior is an added advantage to fight with soil-borne fungal plant pathogens under adverse conditions. They revealed that the *B. subtilis* strain RH5 well colonized the surrounding of the fungal mycelia and inhibition of spore germination, causing cytoplasmic leakage and bifurcation in newly generated hyphae of *R. solani*[33]. Similarly, under laboratory conditions, specific strains of *B. subtilis* and *P. fluorescens* suppressed mycelial growth up to 75% and 51%, respectively[41], while Raj et al.[41] found that the *B. subtilis* Bs-1 isolate demonstrated the highest growth inhibition (60.20%) of *R. solani*. Additionally, both *P. fluorescens* and *B. subtilis* isolates have been shown in numerous studies to be able to inhibit the pathogen's mycelial growth[42] because of their ability to produce certain enzymes, volatile organic compounds, peptides, and antibiotics[43–44]. These bacterial biochemical compositions play a vital role in the control of fungal growth and inhibit its disease causal mechanisms. Therefore, bacterial treatments result in positive effects on plant growth and disease management.

Under field conditions, all the bacterial bioagents showed lesion length reduction and tiller infection reduction over control in BRRI dhan58 at 70 and 81 DAT (Table 2). At 81 DAT the maximum (55.25%) lesion length reduction and (54.80%) tiller infection reduction was recorded in BDISO45JoyR (*B. subtilis* strain SA9). This suggests that these bioagents could potentially reduce sheath blight incidence in rice under specific conditions. Another study investigated the efficacy of *Bacillus* spp. for controlling sheath blight disease in rice where they found that the application of *B. subtilis* significantly reduced the incidence of sheath blight[45]. Previous studies found that these antimicrobial peptides were observed from a wide range of biocontrol active Bacillus species[46–47]. It was assumed that the antimicrobial peptides produced by bacterial species had been responsible for antifungal activity[48]. T_5 [BDISOB221R (*Pseudomonas* sp.)] showed maximum plant height (116.5 cm) followed by BDISO45JoyR (*B. subtilis*) (115.25 cm) compared to positive control (113.25 cm) (Table 3). The bacterial bioagents were used in this study, among them, the plants treated with T_2 [BDISO45JoyR (*B. subtilis*)] showed maximum panicle length, (22.93 cm), number of grains per panicle (234.75), weight of 1,000 grains (g) (22.44 g), yield (5.28 t/ha) and the lowest results were recorded in untreated plants T_0 (Control) (Table 3). The plants treated with T_2 [BDISO45JoyR (*B. subtilis*)] showed the lowest number of chuffy grains per panicle (11.5), and the highest number of chuffy grains per panicle was recorded in untreated plants T_0 (Control) in this study (Table 3). T_2 [BDISO45JoyR (*B. subtilis*)] showed maximum performance due to both it's inherent compositions and better treatment compositions.

The application of bacterial bioagents as a talc-based formulation reduced disease severity and promoted plant growth under field conditions. Though positive control treatment reduced the disease intensity, bacterial bioagent treatments played a dual role by reducing disease severity and promoting the growth of the plant, resulting in increased biomass and yield. A study found that inoculated rice plants with bacterial bioagents resulted in increasing plant height, number of tillers, and grain yield compared to non-inoculated plants[49]. The researchers suggested that the bacteria may have stimulated plant growth by increasing the availability of nutrients or producing growth-promoting compounds. The treatments of rice plants with bacterial bioagents showed significant enhancement of plant height and biomass as compared to

Table 3. Effect of different formulated bacterial bioagents in enhancing growth and yield parameters of rice.

Treatment	Plant height (cm)	Panicle length (cm)	No. of grains per panicle	No. of chaffy grains per panicle	Weight of 1,000 grains (g)	Yield (t/ha)	% Yield increase
T_0	106 ± 2.97c	19.3 ± 0.9d	138.5 ± 1.9e	54.3 ± 2.2a	21.7 ± 0.3b	3.34 ± 0.06e	0e
T_1	113.3 ± 1.7ab	23.2 ± 0.6a	240.3 ± 1.7a	9.8 ± 0.9d	22.7 ± 0.05a	5.87 ± 0.09a	76.29 ± 1.44a
T_2	115.3 ± 2.3ab	22.9 ± 0.6ab	234.8 ± 2.6ab	11.5 ± 1.3cd	22.4 ± 0.1ab	5.28 ± 0.06b	58.51 ± 0.9b
T_3	110.5 ± 1.9abc	21.5 ± 0.6bc	221.8 ± 2.6cd	16.3 ± 1.3b	22.3 ± 0.1ab	4.38 ± 0.09d	31.57 ± 1.39cd
T_4	109 ± 2.5bc	21.2 ± 0.3cd	215.7 ± 2.7de	17.2 ± 0.9b	22.2 ± 0.2ab	4.31 ± 0.11d	29.67 ± 1.65d
T_5	116.5 ± 1.1a	21.9 ± 0.2abc	228 ± 3.2bc	14.8 ± 1.1bc	22.4 ± 0.2ab	4.53 ± 0.09c	36.03 ± 1.49c
Level of significance	0.05	0.05	0.05	0.05	0.05	0.05	0.05
LSD	6.3	2.42	7.66	4.28	1.58	0.21	5.08
CV (%)	3.74	4.63	8.69	13.79	2.32	2.39	8.50

Mean values ± standard error. Values with similar letters are statistically similar. T_0 = Control, T_1 = Positive control, T_2 = BDISO45JoyR (*B. subtilis*), T_3 = BDISO49JoyR (*B. subtilis*), T_4 = BDISOB219R (*P. taiwanensis*), and T_5 = BDISOB221R (*Pseudomonas* sp.).

control. According to Karnwal et al.[50], plots treated with *B. subtilis* UASP17 produced more than the control. Their ability to create IAA, siderophores, atmospheric nitrogen fixation, and solubilize phosphate by both antagonist strains may be the reason, as their employment as biological agents aids in promoting plant growth[51–52]. Similiarly, another study showed that *B. subtilis* strain RH5 produces various plant growth-promoting substances such as hydrogen cyanide, siderophore, indole acetic acid, and has phosphorus, potassium and zinc solubilizing activity, which help plants to survive under *R. solani* infections[53]. IAA promotes plant growth, and also helps plants grow by improving root formation and growth[54]. Although phosphate is a crucial ingredient for plants, it is nevertheless inaccessible in certain forms[55]. Researchers cited several explanations for the consortium's higher efficacy, including the variety of mechanisms antagonists in the collaboration offer, the wide spectrum of target pathogens, and the synergistic relationship between compatible consortium members[56]. In addition to breaking down the cell walls of invasive pathogens, *Pseudomonas fluorescens* and *Bacillus subtilis* are known to produce defense-related enzymes during the early stages of colonisation, including chitinase, peroxidase, and β-1,3-glucanase. These enzymes may activate induced systemic resistance (ISR) in host plants, increasing their resistance to a variety of phytopathogens[52,57]. According to reports, both antagonists contribute to the formation of biofilm[58] between the pathogen and the lesion, which helps to keep the pathogen and host from coming into contact.

In contrast, the use of fungicides is the most popular strategy for controlling rice sheath blight[59], with foliar spraying[60], and seed treatment[61] being the widely adopted application techniques. Fungicides continue to be the most successful control method for rice sheath blight, lowering percent disease index (PDI) ranged from 11.16% to 34.85%, with a reduction in PDI ranging from 53.1% to 67.9%[62]. They prevent the spread of the disease on rice sheaths by affecting *R. solani* and its sclerotia in a number of ways, such as by rupturing the fungal cell membrane[63], inhibiting enzymes[64], interfering with vital functions like respiration or energy production[65–66], or disrupting metabolic pathways linked to the biosynthesis of sterol and chitin for the formation of cell walls[67]. Although this study shows that bacterial bioagents can be used to manage rice sheath blight, its conclusions are limited by a single-year, single-location field experiment. This narrow focus might not adequately take into consideration differences in pathogen dynamics, environmental factors, and rice cultivar reactions. Hence, these bioagents require additional multi-location and multi-year tests to ensure their resilience and wider applicability. Since the initial experiment focused on evaluating the effects of individual bioagents against *R. solani* to manage rice sheath blight, future studies could explore their combined application to assess potential synergistic effects and improved disease control. Given the strong evidence for bacterial bioagents in sheath blight control, further efforts should focus on their formulation and large-scale commercialization. Additionally, characterizing biosynthetic gene clusters responsible for antifungal secondary metabolites could lead to the development of novel biopesticides for managing this seed- and soil-borne pathogen.

Conclusions

The results of the dual culture assay revealed that among four bacterial bioagents of different species, BDISO45JoyR (*B. subtilis*) was the most effective against *R. solani*, exhibiting 76.89% inhibition of radial mycelial growth. The results showed that the application of BDISO45JoyR (*B. subtilis*) significantly reduced the lesion length by 55.25% compared to the untreated control in field experiments. Similarly, the same bacterial bioagent also reduced the maximum tiller infection by 54.80. Furthermore, BDISOB221R (*Pseudomonas* sp.) showed a maximum plant height of 116.5 cm. From all treatments, BDISO45JoyR (*B. subtilis*) resulted in the maximum yield of 5.28 t/ha (58.51% increase over control) compared to the control. These findings suggest that the use of these bacterial bioagents can serve as a promising eco-friendly approach for the management of rice sheath blight, contributing to sustainable rice production. However, the evaluation of the field potential of these bacterial bioagents in other rice varieties in different seasons will be the next step of the research, to find out the best potential bacterial bioagents in controlling sheath blight of rice. In addition, identification of the bioactive compound(s) from these bacterial bioagents and formulation of biopesticide(s) instead of live bacteria for field application would be the best option for future studies.

Author contributions

The authors confirm contribution to the paper as follows: data collection: Reedoy MAH, Khan I, Sarly SP, Rahaman M, Dipty AR; laboratory work, and data analysis: Reedoy MAH; analysis and interpretation of results: Reedoy MAH, Shahi M, Hasan MH, Auyon ST, Islam MR; draft manuscript preparation: Reedoy MAH, Shimu JF, Hasan MH, Singha UR; project supervison: Islam MR. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

This research was carried out with the financial support from National Agricultural Technology Phase-2.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/tia-0025-0005)

Dates

Received 19 October 2024; Revised 17 March 2025; Accepted 29 March 2025; Published online 2 July 2025

References

1. United States Department of Agriculture (USDA). 2020. *Rice sector at a glance*. (Retrieved 2024 March 10). www.ers.usda.gov/topics/crops/rice/rice-sector-at-a-glance/
2. Samal P, Babu SC, Mondal B, Mishra SN. 2022. The global rice agriculture towards 2050: an inter-continental perspective. *Outlook on Agriculture* 51:164–72
3. Bangladesh Bureau of Statistics. 2022. *Yearbook of Agricultural Statistics*. Bangladesh Bureau of Statistics, Bangladesh
4. Khan MAR, Mahmud A, Ghosh UK, Hossain MS, Siddiqui MN, et al. 2023. Exploring the phenotypic and genetic variabilities in yield and yield-related traits of the diallel-crossed F5 population of aus rice. *Plants* 12:3601

5. FAOSTAT. 2022. *Crops and Livestock products domains*. FAO, Rome
6. Sarkar MAR, Rahman MC, Rahaman MS, Sarker MR, Islam MA, et al. 2022. Adoption determinants of exotic rice cultivars in Bangladesh. *Frontiers in Sustainable Food Systems* 6:813933
7. Zhao M, Lin Y, Chen H. 2020. Improving nutritional quality of rice for human health. *Theoretical and Applied Genetics* 133:1397–413
8. Ou SH. 1985. *Rice diseases*, 2nd edition. Commonwealth Mycological Institute, Kew
9. Savary S, Castilla NP, Elazegui FA, McLaren CG, Ynalvez MA, et al. 1995. Direct and indirect effects of nitrogen supply and disease source structure on rice sheath blight spread. *Phytopathology* 85:959–65
10. Islam AKMS, Bhuiyan R, Khan MAI, Akter S, Islam MR, et al. 2025. Synergistic antifungal activity of green synthesized zinc oxide nanoparticles and fungicide against *Rhizoctonia solani* causing rice sheath blight disease. *Applied Biochemistry and Biotechnology* 197:587–612
11. Senapati M, Tiwari A, Sharma N, Chandra P, Bashyal BM, et al. 2022. *Rhizoctonia solani* Kühn pathophysiology: status and prospects of sheath blight disease management in rice. *Frontiers in Plant Science* 13:881116
12. Li D, Tang Q, Zhang Y, Qin J, Li H, et al. 2012. Effect of nitrogen regimes on grain yield, nitrogen utilization, radiation use efficiency, and sheath blight disease intensity in super hybrid rice. *Journal of Integrative Agriculture* 11:134–43
13. Chithrashree, Udayashankar AC, Chandra Nayaka S, Reddy MS, Srinivas C. 2011. Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *Biological Control* 59:114–22
14. Boukaew S, Klinmanee C, Prasertsan P. 2013. Potential for the integration of biological and chemical control of sheath blight disease caused by *Rhizoctonia solani* on rice. *World Journal of Microbiology and Biotechnology* 29:1885–93
15. Singh P, Mazumdar P, Harikrishna JA, Babu S. 2019. Sheath blight of rice: a review and identification of priorities for future research. *Planta* 250:1387–407
16. Yellareddygari SKR, Reddy MS, Kloepper JW, Lawrence KS, Fadamiro H. 2014. Rice sheath blight: a review of disease and pathogen management approaches. *Journal of Plant Pathology & Microbiology* 5:4
17. Panda N, Heinrichs EA, Hibino H. 1984. Resistance of the rice variety Utri Rajapan to ragged stunt and tungro viruses. *Crop Protection* 3:491–500
18. Kannaiyan S, Prasad NN. 2023. Effect of foliar spray of certain fungicides on the control of sheath blight disease of rice. *Madras Agricultural Journal* 71:111–14
19. Monsur MA, Biñas SC, Herath SN, Ambita IDV, Tasnim Z, et al. 2023. Comparative study between biological and chemical agents for control sheath blight disease of rice. *Journal of Plant Stress Physiology* 9:10–17
20. Islam R, Nihad SAI, Obaidullah AJM, Al Mamun A, Moniruzzaman M, et al. 2021. Efficacy of biological, chemical and cultural practices for the management of foot and root rot disease of black cumin. *Biocatalysis and Agricultural Biotechnology* 37:102193
21. Fang L, Liao X, Jia B, Shi L, Kang L, et al. 2020. Recent progress in immunosensors for pesticides. *Biosensors and Bioelectronics* 164:112255
22. Fernández-Ortuño D, Pérez-García A, López-Ruiz F, Romero D, de Vicente A, et al. 2006. Occurrence and distribution of resistance to QoI fungicides in populationsof *Podosphaera fusca* in south central Spain. *European Journal of Plant Pathology* 115(2):215–22
23. Nagórska K, Bikowski M, Obuchowski M. 2007. Multicellular behaviour and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. *Acta Biochimica Polonica* 54(3):495–508
24. Khare A, Singh BK, Upadhyay RS. 2010. Biological control of *Pythium aphanidermatum* causing damping-off of mustard by mutants of *Trichoderma viride*. *Journal of Agricultural Technology* 6:231–43
25. Howell CR. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease* 87:4–10
26. Nagarajkumar M, Bhaskaran R, Velazhahan R. 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. *Microbiological Research* 159:73–81
27. Somani AK, Arora RK. 2010. Field efficacy of *Trichoderma viride, Bacillus subtilis*, and *Bacillus cereus* in consortium for control of *Rhizoctonia-solani* causing sheath blight disease of rice. *Indian Phytopathology* 63(1):23–25
28. Pérez-Montaño F, Alías-Villegas C, Bellogín RA, del Cerro P, Espuny MR, et al. 2014. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. *Microbiological Research* 169:325–36
29. Radja Commare R, Nandakumar R, Kandan A, Suresh S, Bharathi M, et al. 2002. *Pseudomonas fluorescens* based bio-formulation for the management of sheath blight disease and leaffolder insect in rice. *Crop Protection* 21(8):671–77
30. Cavaglieri L, Orlando J, Rodríguez MI, Chulze S, Etcheverry M. 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in vitro* and at the maize root level. *Research in Microbiology* 156:748–54
31. White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols*, eds Innis MA, Gelfand DH, Sninsky JJ, White TJ. US: Academic Press. pp. 315–22. doi: 10.1016/b978-0-12-372180-8.50042-1
32. Rahman MM, Masud MM, Iqbal Hossain M, Islam NET, Alam MZ, et al. 2021. Potential role of rice plant growth promoting phylloplane and rhizospheric bacteria in controlling *Xanthomonas oryzae* pv. *oryzae*. In *Integrative Advances in Rice Research*, ed. Huang M. London: IntechOpen. doi: 10.5772/intechopen.99854
33. Ooi YS, Nor NMIM, Furusawa G, Tharek M, Ghazali AH. 2022. Application of bacterial endophytes to control bacterial leaf blight disease and promote rice growth. *The Plant Pathology Journal* 38(5):490–502
34. Dutta P, Deb L. 2020. An innovative technique for artificial inoculation of *Rhizoctonia solani* Kuhn for field experiments. *International Journal of Current Microbiology and Applied Sciences* 9:1077–85
35. Kumar M, Giri VP, Pandey S, Gupta A, Patel MK, et al. 2021. Plant-growth-promoting rhizobacteria emerging as an effective bioinoculant to improve the growth, production, and stress tolerance of vegetable crops. *International Journal of Molecular Sciences* 22:12245
36. Gónzalez D, Rodriguez-Carres M, Boekhout T, Stalpers J, Kuramae EE, et al. 2016. Phylogenetic relationships of *Rhizoctonia* fungi within the Cantharellales. *Fungal Biology* 120(4):603–19
37. Yu Y, Gui Y, Li Z, Jiang C, Guo J, et al. 2022. Induced systemic resistance for improving plant immunity by beneficial microbes. *Plants* 11:386
38. Upadhyay BK, Dubey SC, Singh R, Tripathi A. 2015. Diversity analysis and comparison of ITS and SCAR-based molecular markers to detect *Rhizoctoniasolani* using conventional and real-time PCR. *Journal of Pure and Applied Microbiology* 9(2):1281–93
39. Sandoval RFC, Cumagun CJR. 2019. Phenotypic and molecular analyses of *Rhizoctonia* spp. associated with rice and other hosts. *Microorganisms* 7(3):88
40. Nur Mawaddah S, Mohd Zafri AW, Sapak Z. 2023. The potential of *Pseudomonas fluorescens* as biological control agent againstsheath blight disease in rice: a systematic review. *Food Research* 7:46–56
41. Nagendran K, Karthikeyan G, Peeran MF, Raveendran M, Prabakar K, et al. 2013. Management of bacterial leaf blight disease in rice with endophytic bacteria. *World Applied Science Journal* 28(12):2229–41
42. Suthin Raj T, Muthukumar A, Renganathan P, Suji HA. 2020. Efficacy of seaweed and seawater associated *Bacillus velezensis* against sheath blight of rice caused by *Rhizoctonia solani* Kuhn. *Plant Archives* 20:3727–31
43. Safdarpour F, Khodakaramian G. 2019. Assessment of antagonistic and plant growth promoting activities of tomato endophytic bacteria in challenging with *Verticillium dahliae* under *in-vitro* and *in-vivo* conditions. *Biological Journal of Microorganism* 7:77–90
44. Carmona-Hernandez S, Reyes-Pérez JJ, Chiquito-Contreras RG, Rincon-Enriquez G, Cerdan-Cabrera CR, et al. 2019. Biocontrol of postharvest fruit fungal diseases by bacterial antagonists: a review. *Agronomy* 9:121
45. Harish S, Kavitha K, Singh AK. 2014. Biocontrol potential of *Bacillus subtilis* against neck blast disease of rice caused by *Magnaporthegrisea*. *Journal of Biological Control* 28(4):220–27
46. Chen X, Zhang Y, Fu X, Li Y, Wang Q. 2016. Isolation and characterization of *Bacillus amyloliquefaciens* PG12 for the biological control of apple ring rot. *Postharvest Biology and Technology* 115:113–21

47. Sharma V, Kumar V, Kumar B, Singh R. 2018. Evaluation of *Bacillus aerophilus* for control of rice blast disease under field conditions. *Archives of Phytopathology and Plant Protection* 51(3–4):195–202
48. Zhang L, Sun C. 2018. Fengycins, cyclic lipopeptides from marine *Bacillus subtilis* strains, kill the plant-pathogenic fungus *Magnaporthe grisea* by inducing reactive oxygen species production and chromatin condensation. *Applied and Environmental Microbiology* 84:e00445-18
49. Kaur G, Reddy MS, Kumar S. 2018. *Stenotrophomonas maltophilia* inoculation enhances rice growth and grain yield under field conditions. *Plant Growth Regulation* 86(2):293–301
50. Karnwal A, Mannan M. 2018. Application of *Zea mays* L. Rhizospheric bacteria as promising biocontrol solution for rice sheath blight. *Pertanika Journal of Tropical Agricultural Science* 41(4):1705–20
51. Jamali H, Sharma A, Roohi, Srivastava AK. 2020. Biocontrol potential of *Bacillus subtilis* RH5 against sheath blight of rice caused by *Rhizoctonia solani*. *Journal of Basic Microbiology* 60:268–80
52. Nascimento FX, Urón P, Glick BR, Giachini A, Rossi MJ. 2021. Genomic analysis of the 1-aminocyclopropane-1-carboxylate deaminase-producing *Pseudomonas thivervalensis* SC5 reveals its multifaceted roles in soil and in beneficial interactions with plants. *Frontiers in Microbiology* 12:752288
53. Radhakrishnan R, Hashem A, Abd Allah EF. 2017. *Bacillus*: a biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology* 8:667
54. Rawat P, Shankhdhar D, Shankhdhar SC. 2022. Synergistic impact of phosphate solubilizing bacteria and phosphorus rates on growth, antioxidative defense system, and yield characteristics of upland rice (*Oryza sativa* L.). *Journal of Plant Growth Regulation* 41:2449–61
55. Niu B, Wang W, Yuan Z, Sederoff RR, Sederoff H, et al. 2020. Microbial interactions within multiple-strain biological control agents impact soilborne plant disease. *Frontiers in Microbiology* 11:585404
56. Poirier Y, Jaskolowski A, Clúa J. 2022. Phosphate acquisition and metabolism in plants. *Current Biology* 32:R623–R629
57. Pandit A, Adholeya A, Cahill D, Brau L, Kochar M. 2020. Microbial biofilms in nature: unlocking their potential for agricultural applications. *Journal of Applied Microbiology* 129(2):199–211
58. Haque Z, Khan MR. 2021. Identification of multi-facial microbial isolates from the rice rhizosphere and their biocontrol activity against *Rhizoctonia solani* AG1-IA. *Biological Control* 161:104640
59. Kandhari J, Gupta RL. 2003. Efficacy of fungicides and resistance inducing chemicals against sheath blight of rice. *Journal of Mycopathological Research* 41:67–69
60. McGrath MT. 2004. What are fungicides. *The Plant Health Instructor* 5:2
61. Kabir MH, Islam SM, Sultana N, Azad MA, Fakir GA. 2006. Effect of seed cleaning, washing and treating with Vitavax on incidence and severity of Boro rice diseases. *International Journal of Sustainable Agricultural Technology* 2:27–31
62. Sharma S, Tripathi SK, Sharma AK, Prajapati S, Gautam V, et al. 2024. Evaluation of chemical fungicides against sheath blight disease of rice in India. *International Journal of Plant & Soil Science* 36:177–83
63. Croucher L, Jewess P. 1999. *Metabolic pathways of agrochemicals: insecticides and fungicides*. Great Britain: The Royal Society of Chemistry. pp. 1134–37. doi: 10.1039/9781847551375
64. Kumar P, Ahlawat S, Chauhan R, Kumar A, Singh R, et al. 2018. *In vitro* and field efficacy of fungicides against sheath blight of rice and post-harvest fungicide residue in soil, husk, and brown rice using gas chromatography-tandem mass spectrometry. *Environmental Monitoring and Assessment* 190:503
65. Ichiba T, Kumano K, Kashino H, Nanba K, Mizutani A, et al. 2000. Effect of metominostrobin on respiratory activity of *Rhizoctonia solani* and its efficacy for controlling rice sheath blight. *Journal of Pesticide Science* 25:398–401
66. Lal M, Sharma S, Chakrabarti SK, Kumar M. 2017. Thifluzamide 24% SC: a new molecule for potato tubers treatment against black scurf disease of potato caused by *Rhizoctonia solani*. *International Journal of Current Microbiology and Applied Sciences* 6:370–75
67. Morton V, Staub T. 2008. A short history of fungicides. *APSnet Feature Articles*. www.apsnet.org/edcenter/apsnetfeatures/Pages/Fungicides.aspx