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Rhizosphere Bacteria Isolated from Medicinal Plants Improve Rice Growth and Induce Systemic Resistance in Host Against Pathogenic Fungus

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Abstract

Sheath blight (*ShB*) disease is a major biotic stress that causes significant yield loss in rice. Plant growth-promoting rhizobacteria (PGPRs) have been found to suppress the adverse effect of disease on plants. In the present investigation, an attempt has been made to evaluate the effect of PGPR strains isolated from the rhizosphere soil of medicinal plants on rice under stress conditions. We isolated 158 morphologically distinct bacterial strains and tested them against *R. solani* under *in-vitro* conditions and found 52 promising strains with more than 50% antifungal activity. These strains were examined for their physiological and biochemical characteristics and further confirmed with 16S rDNA gene-specific markers. Strains that inflicted > 80% inhibition during *in-vitro* studies were selected for pot and field experiments. The results indicated that *Bacillus velezensis*, *B. megaterium*, and *B. toyonensis* registered significantly higher plant growth-promoting activities with enhanced germination, seedling vigor, and dry weight. In addition, applying these PGP strains exhibits the lowest disease incidence, relative lesion length, delayed sclerotia formation, and recorded maximum grain yield per pot. The field study further confirmed that *B. toyonensis* provided significant disease suppression with least disease incidence (PDI: 17.37 and 12.88), relative lesion length percent (27.71and12.88), area under disease progress curve (382.98 and 286.25) value (AUDPC), and highest grain yield (63.00 and 48 t ha^{-1}) in Tapaswini and CR Dhan 1014 varieties, respectively, followed by *B. megaterium and B. velezensis*. The PGPR-treated plants also showed enhanced activities of defense enzymes like polyphenol oxidase, superoxide dismutase, and catalase showing induced systemic resistance (ISR). Thus, these three PGPR strains from medicinal plants enhanced the tolerance of rice to *ShB* disease with improved crop growth. Integrating these PGPR in seed treatment, seedling root dip and foliar application will improve the rice yield and farmers' livelihood.

Keywords Sheath blight · Biocontrol · PGPR · *Rhizoctonia solani* · *Bacillus*

Introduction

Rice (*Oryza sativa* L.) is the prime staple food crop of about half of the global population. The crop is cultivated in diverse ecologies, particularly in Asia, where it occupies more than 70% of the arable land (Pathak et al. 2018). Under changing climatic conditions, rice farming faces more challenges from biotic and abiotic stresses (Yadav et al. 2018). Among the biotic stresses encountered by rice, pathogens create diseases that are a season-long problem due to damage at different stages of the crop (Raghu et al. 2020). Major diseases of rice like blast, brown spot, bacterial blight, sheath blight, and rice tungro virus continue to cause severe damage and losses (Jacobs and Wang 2021), and minor diseases like false smut, sheath rot, bakanae, early seedling blight, narrow brown spot, and grain discoloration have emerged as a serious problem in recent years (Raghu et al. 2018). Sheath blight being caused by *Rhizoctonia solani* Kuhn (AG-1) infects crops in almost all ecologies. Under favorable conditions, soil-borne sclerotia germinate to form

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mycelia. Later mycelium establishes contact with the rice plant surface and grows. Same time, it produces infection structures like infection cushions and lobate appressoria. This appressorium helps mycelia penetration into the host tissue. In some cases, the infection may also occur through natural openings such as stomata (Marshall et al. 1980). The pathogen can spread both vertically and horizontally with 20 cm/day of horizontal spread under favorable conditions (Savary et al. 1995).

Various kinds of management options are available to manage biotic stresses. However, farmers depend heavily on chemical pesticides to get quick results (Guru-Pirasanna-Pandi et al. 2021). On the other hand, intensive use of chemical fertilizers has created numerous environmental problems like soil and water pollution, ill effects on human and animal health, the evolution of new biotypes/strains/races, and the development of pesticide resistance (Swain et al. 2018). Therefore, there is a necessity for safer, more efficient, and eco-friendly management measures for mitigating biotic stresses. One such attractive and promising approach is using biological control agents (Shoresh et al. 2010). These biocontrol agents can be exploited for their multi-trait characteristics to enhance plant growth, pathogen suppression, and induced disease resistance (Dutta et al. 2010; Mukherjee et al. 2014). They can be found in the nutrient-rich rhizosphere, rhizoplane, phyllosphere, and phylloplane (Hartman et al. 2009; Molla et al. 2012).

Plant growth-promoting rhizobacteria (PGPR) represent a wide group of rhizosphere-inhabiting bacteria that can colonize plant roots, and above-ground parts, and help in the stimulation of plant growth by direct or indirect activities (Beneduzi et al. 2012; Dinesh et al. 2014). These beneficial bacteria' direct mechanisms include biofertilization, plant growth stimulation, rhizoremediation, and plant stress (biotic or abiotic) control (Beneduzi et al. 2012; Dinesh et al. 2014). These PGPR can be isolated from various sources like the rhizosphere of cultivated plants, forest trees, medicinal plants, etc. PGPR suppresses the disease/disease-causing microbes through antibiosis, induction of systemic resistance, and competition for nutrients and habitats (Lugtenberg and Kamilova 2009). In the current era of disease management, PGPR-mediated strategies have gained prominence because they are cost-effective, non-hazardous to the application, pollution free, residue free, and result in cleaner crop production. Overall, biological control through PGPR can enhance plant growth and crop yields by efficiently checking disease pressure (Adesemoye and Kloepper 2009; Van Loon and Bakker 2009). Several root-associated (PGPR) bacterial species have shown their efficacy for plant growth promotion and disease control viz., *Acinetobacter, Alcaligenes, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Pseudomonas, Rhizobium,* and *Serratia* (Anandaraj and Dinesh 2008). PGPR is being used on a larger scale, either alone or in consortia with biofertilizers and other strains of bacteria to improve their efficacy. Among different crop species, medicinal plants harbor a distinctive microbiome due to their unique and structurally divergent bioactive secondary metabolites specific to associated microorganisms (Qi et al. 2012). Medicinal plants are rich in secondary metabolites and potentially useful for producing natural drugs (Thirumurugan et al. 2018; Senthil-Nathan et al. 2022). Thus, these plant species support a great diversity of beneficial microflora in their rhizosphere, including plant growth-promoting rhizobacteria (PGPR) (Kaul et al. 2022; Devi et al. 2023). Thus, evaluating these PGPR for rice antifungal characters may help to manage most diseases efficiently (Raajimakers et al. 2009). With this information, the present study was undertaken with the objectives to isolate and characterize the PGPR from the rhizosphere soil of medicinal plants, to test their efficacy against *Rhizoctonia solani*, causing sheath blight disease, and to understand the mechanism of induced systemic resistance (ISR) and plant growth promotion.

Material and Methods

Soil Sampling

For isolation of PGPR, soil samples were collected from the rhizosphere of different medicinal plants [*Abrus precatorius, Acorus calamus, Aegle marmelos, Aegle marmelos, Allium cepa, Aloe vera, Argyreia nervosa, Asparagus racemosus, Atalantia monophylla, Azadirachta indica, Celastrus paniculata, Centella asiatica, Cinnamomum zeylanicum, Cissus quadrangularis, Citrus sinensis, Costus speciosus, Crinum asiaticum, Curcuma longa, Cymbopogon martinii, Ficus hispida, Garcinia cowa, Gymnema sylvestre, Madhuca indica, Mallotus philippensis, Momordica charantia, Mucuna monosperma, Nelsonia canescens, Nyctanthes arbor tristis, Ocimum basillum, Ocimum canum, Ocimum citriodorum, Ocimum gratissimum, Ocimum sanctum, Ocimum tenuiflorum, Paederia foetida, Phyllanthus acidus, Piper longum, Pongamia pinnata, Rauvolfia serpentina, Saraca asoca, Sansevieria roxburghiana, Solanum torvum,* and *Oryza sativa*] in 15 locations of Cuttack, Bhadrak, Balasore, and Jagatsinghpur districts of Odisha (India). The details of the sampling site (GPS coordinates) and sample code are provided in Supplementary Table 1. The soil adhering to the roots of medicinal plants was collected according to the standard procedure (Garcia et al. 2005; Dinesh et al. 2014). Random soil sampling was done and the soil was collected in polyethylene zip-lock bags (250–500 g capacity) (Purchased from a local seller; M/S Science cell, Cuttack) and immediately transferred to an ice box and transported to the laboratory of ICAR-National Rice Research Institute (NRRI), Cuttack for

further analysis. The soil sample had living plant material, coarse roots, and some inert matter. They were cleaned and stored at 4 °C before analysis of microbial parameters and biochemical tests. For all the laboratory analysis, molecular grade chemicals were obtained from Himedia, Sigma-Aldrich, MP Biomedicals, and Merck Mumbai, India.

Isolation, Enumeration, and Characterization of PGPR

From the stored sample, exactly 10.0 g of moist soil was drawn, placed in a 90 mL conical flask (10^{-1}), and continuously shaken in an incubator shaker for 10 min (Weller and Cook 1983). Then, serial dilutions were made from this stock solution. Exactly 1.0 mL suspension was transferred from a 90 mL stock solution into a 9 mL blank (10^{-2}) in a test tube. The serial dilution was continued up to 10^{-10} dilutions. Pour plating techniques were used for isolation, where 1.0 mL of the diluents from each tube was transferred to Petri plates (90.0 mm), followed by pouring of molten media of Tryptic Soy Agar (TSA), Kings-B Agar, and Nutrient Agar (NA) medium, separately. The basal medium was amended with glucose, mannitol, sorbitol, inositol, and sucrose as suggested by Dinesh et al. (2014). The colony population and suitable dilution were selected further for selecting individual colonies, estimating the population of rhizobacteria, and expressing the number as colony forming units (CFU) g^{-1} of soil. Single colonies with different morphological characteristics were selected and sub-cultured on nutrient agar. A total of 158 strains were obtained from an individual selection from all the locations. Each strain was provided with a unique identification code in CRM series from CRM-1 to CRM-158. These strains were kept at − 80 °C in a deep freeze in 40% glycerol until further studies. All the strains were studied for their morphological (cell form, colony color, appearance, spore formation, and size) and biochemical (Gram's staining, acid production, gelatin liquefaction, indole production, motility, production of UV-fluorescent pigments, methyl red test, Voges–Proskauer (VP) test, citrate test, catalase test, ammonia production, and starch hydrolysis) characteristics before testing their antifungal activities (Dinesh et al. 2014). Other tests like the growth of PGPR at different temperatures (28 °C, 32 °C, 35 °C, 37 °C, 41 °C, and 50 °C), salt concentrations (1%, 2%, 5%, 7%, and 10% of NaCl), pH concentrations (4, 5, 6, 7, and 8) were also studied.

Isolation and Characterization of Pathogen

Rhizoctonia solani was isolated from infected tissue of the highly susceptible rice variety Tapaswini collected from a farmer's field (20.3124° N, 85.8691° E). A standard tissue isolation procedure was followed and pure cultures were isolated on potato dextrose agar (PDA) plates amended with 0.1% chloramphenicol (). The fungal strains were characterized morphologically and DNA sequencing analysis using ITS primers (ITS-1; F-5′-TCCGTAGGTGAACCTGCGG-3′; ITS-4; R-5′-TCCTCCGCTTATTGATATGC-3′). The isolated strain was subjected to a pathogenicity test by following Koch's postulates. For this purpose, four-day-old pure culture of the pathogen was sub-cultured on PDA and mass multiplied on rice straw and PDB for 10–14 days. The pathogen was then inoculated on 45-day-old (maximum tillering to the booting stage) rice plants of the Tapaswini cultivar by placing inoculum at the center of the tiller and tying the tiller bunch with thread. Inoculated seedlings were maintained at greenhouse at 27 °C ± 2, and observable disease symptoms were recorded after 6 days of inoculation. The pathogen was re-isolated and compared with the original strain to satisfy Koch's postulates.

Screening and Identification of Antagonistic Bacteria

All 158 PGPR strains were subjected to an *in-vitro* confrontation assay using a dual-plate technique (Berg et al. 2002). The bacterial strains were streaked on either side of the potato dextrose agar (PDA) medium. Actively growing, 4-day-old test pathogens were placed exactly at the center of the media (around 2–3 cm from the bacteria). The plates were incubated at 28 °C for about 5–6 days or until the complete growth of the test pathogen in control plates. Three replications of each bacterial strain were maintained. The radial growth of the fungal mycelium was measured once the growth in control plates touches the edge of the plates. The percent inhibition of the pathogen growth was estimated. The following formula measured the percent inhibition of the pathogen.

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

where C is Radial growth of the pathogen in the control plate, T is Radial growth of the pathogen in a treated plate. The rhizobacterial strains, which showed more than 80% of pathogen inhibition, were taken for further studies.

Morpho-Physiological and Molecular Characterization of PGPR Strains

A total of 52 promising PGPR strains were selected for morphological, biochemical, and molecular characterization. The methodology explained in the previous section was followed with appropriate modifications (Holt et al. 1994; Tindal et al. 2007). DNA sequencing and phylogenetic analysis were carried out to identify the strains at the molecular level. The genomic DNA was isolated from all

52 strains using a standard protocol (Dinesh et al. 2014). The purified DNA was subjected to PCR amplification using the 16 s rDNA gene primer set pA (5′-AGAGTTTGATCC TGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCG CA-3′). A PCR reaction was carried out in 20 µl of reaction mixture containing 2 µl of 1X buffer (10 mM Tris pH9; 50 mM KCl, 0.01% gelatin), 3 mM $MgCl_2$, 2 µl of 2 mM dNTP mixture, forward and reverse primers 2 µl each, 1 µl of 3 U Taq DNA polymerase, 8 µl of nanopore water, and 1 µl of 100 ng template DNA. The PCR reaction was performed in a thermocycler (BioRad USA, T-100) with the following conditions: initial denaturation at 94 °C for 4 min, 45 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 45 s, polymerization at 72 °C for 2 min, and a final extension step of 72 °C for 5 min. The PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide (0.5 $\mu g ml^{-1}$) and documented using a gel documentation system (BioRad, USA). The PCR product was purified using the QIA quick gel extraction kit (Qiagen, Inc. Chatsworth, California) according to the manufacturer's instructions. The purified product was sent for DNA sequencing at AgriGenome India Pvt. Ltd, Cochin, India. The sequence analysis was performed at NCBI (BLASTn) program and confirmed for the species (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and submitted to NCBI database (MT772272-76, MT774102-12, MT775459-63, MT772260-61, OR453080, OR453082, OR453174, OR453176, OR453181, OR453180, OR453185). The ClustalW program was used to align the 16 s rDNA gene sequences. The aligned sequences were subjected to BLAST search in the NCBI database. The sequences with maximum similarities were retrieved from the NCBI database and phylogenetic analysis was performed using Mega 6.06 software with 1000 bootstrap repetitions (Tamura et al. 2011). The relationship between the isolates and the different species studied was established by constructing a cladogram.

Similarly, the defense-related enzymes were detected in treated plants by using specific markers. In brief, the genomic DNA of all the bacterial strains was subjected to PCR analysis to detect antibiotic biosynthesis genes. The gene-specific primers such as *fenB* for Fengycin (F-5′-CCT GGAGAAAGAATATACCGTACCY & R-5′-GCTGGT TCAGTTKGATCA CA), *ituD* for Iturin (F-5′-TTGAAY GTCAGYGCSCCTTT & R-5′-TGCGMAA ATAAT GGSG TCGT), *srfA* for Surfactin (F- 5'-AGAGCACATTGAGCG TTACAAA & 5′-CAG CAT CTC GT TCAACTTTCAC) were used for the PCR detection (Chung et al. 2008).

Evaluation for Plant Growth Promotion and Disease-Suppressing Activities

Roll Towel Method

For the present study, we took one susceptible (Tapaswini) and one moderately resistant (CR-1014) variety against *R. solani*. The growth-promoting activity of selected PGPR strains was assessed based on the seedling vigor index of rice seeds using the standard roll towel method (ISTA 1993). For each strain, one hundred bacterized seeds (strains were inoculated into LB broth and kept at room temperature with continuous shaking for 48 h at 28 ± 1 °C and 120 rpm) were kept over pre-soaked germination paper at equidistance. Then the paper was covered using another pre-soaked paper and gently rolled. The role was covered by a polythene sheet and incubated in a plant growth chamber at 28 ± 1 °C for 10 days. Each treatment was replicated thrice. The growth parameters like percent germination, root length, shoot length, plant biomass (dry and wet), and vigor index were calculated. The growth parameters such as percent germination, root length, shoot length, plant biomass (dry and wet), and vigor index were calculated as per ISTA protocol.

Pot Experiments

The 48-h-old culture was centrifuged at 5000 rpm for 20 min. The pellet was separated and re-suspended in sterile water. The concentration was adjusted to approximately 10^8 CFU/mL using a spectrophotometer (OD at 595 = 0.30). This bacterial inoculum was used for seed treatment and seedling dip treatments (Thompson et al. 1996). Rice seeds weighing 10 g were placed in zip-lock polythene bags and thoroughly mixed with bacterial suspension for 30 min. The treatment details are as follows: T-1:CRM-13 (*Bacillus velezensis*), T-2:CRM-18 (*Bacillus megaterium*), T-3: CRM-72 (*Bacillus toyonensis*), T-4:CRM-105 (*Paenibacillus cucumis*), T-5:CRM-114 (*Paenibacillus silviae*), T-6:CRM-116 (*Paenibacillus ginsengarvi*), T-7:CRM-133 (*Bacillus megaterium*), T-8:CRM-149 (*Bacillus subtilis*), T-9:CRM-152 (*Bacillus megaterium*), T-10:CRM-156 (*Bacillus cereus*), T-11: Fungicide (Tebuconazole + Trifloxystrobin—75 WG), control, T-12: Untreated check. The bacterized seeds were shade dried and sowed in the earthen pots (12-inch X 9-inch) with the potting mixture containing soil and FYM in a 2:1 ratio. The soil properties of the experiment are as follows. The texture is sandy clay loam with 52% sand, 30% clay, and 18% silt. The bulk density was 1.41 Mg m^{-3}, electrical conductivity was 0.60 dSm^{-1}, and pH (using 1:2.5, soil: water suspension of 0–15 cm depth) was 6.8. The total organic C was 7.4 g kg^{-1}, available N was 0.69 g kg^{-1}, available P was 23.3 mg kg^{-1}, available K content was 276 mg kg^{-1} and the water was maintained at field capacity. The pots were observed daily for germination and

seedling establishment. Control treatment on the other hand, was maintained without bacterial inoculation and all the treatments were replicated thrice for each treatment. The growth parameters like seedlings' root length and shoot length and the percent germination were measured after 12 days of incubation in the greenhouse at 28 ± 1 °C with 85% relative humidity were measured along with germination percent. The following formula calculated seedling vigor (Abdul-Baki and Anderson 1973).

$$\text{Vigor index} - \text{I} = (\text{Mean Root length} + \text{Mean Shot length}) \times \text{germination (\%)}$$

$$\text{Vigor Index} - \text{II} = \text{Germination (\%)} \times \text{Seedling dry weight (mg)}$$

Pathogen Inoculation, Recording Disease Incidence, and Estimation of Plant Defense Enzymes

Two sets of experiments were carried out under pot conditions. The first experiment dealt with the growth promotion activities of promising PGPR strains, and the second experiment assessed the biocontrol potential of selected PGPR strains against sheath blight diseases of rice. The methods for the first experiment are explained in Sect. "Evaluation for plant growth promotion and disease-suppressing activities". The pathogens were multiplied in the laboratory by following standard protocols for biocontrol experiments. *Rhizoctonia solani* culture (*Shb-4*) was mass multiplied on rice straw medium and incubated for 15 days, followed by inoculation to 43-day-old rice plants by placing the straw bit in between the tillers and tied with thread. Standard controls were maintained without pathogen inoculation. The disease incidence was recorded by following the IRRI standard evaluation system (SES) scale (IRRI 2013). The plant defense enzymes like peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) were estimated in all the treatments following standard procedures (Hammerschimidt et al. 1982; Van Rossun et al. 1997; Havir and McHale 1987). The enzyme activity was expressed as the increase in absorbance at respective wavelength min^{-1} mg^{-1} of protein.

Field Experiment Using Promising PGPR For Disease Suppression Activities

The field experiment was conducted in July-November 2019 with promising PGPR strains, chemicals (fungicide), and control. The experiment was laid out in a randomized block design (RBD) with three replications. Highly susceptible variety Tapaswini was transplanted in 5 × 5 M^2 plots maintaining 20 × 15 cm of plant spacing. Water and nutrient management were done as per standard cultivation practices. Following standard procedures, liquid formulations were used for the seedling dip and foliar spray. The disease incidence in treatment plots and control was measured. The grain yield and plant biomass yield from all the treatments were recorded.

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of Infected tillers in each replication}}{\text{Total Number of tillers examined in each replication}} \times 100$$

The percent vertical spread of the disease was calculated by following the formula (Anonymous 2000).

$$\text{Relative Lesion Height (\%)} = \frac{\text{Lesion height of the seedling (cm)}}{\text{Total length of the seedling above the soil (cm)}} \times 100$$

The area under disease progress curve (AUDPC) from each treatment was calculated using the following formula (Wilcoxon et al. 1975).

$$\text{E. AUDPC (A)} = \sum_{\text{i}-1}^{k} 1/2(\text{Si} + \text{Si} - 1) \times \text{d}$$

where, A = AUDPC value, Si = Disease severity at the end of the week i, k = Number of successive disease evaluations, and d = Interval between two evaluations.

Statistical Analysis

The significance of the treatment effects was determined by one-way ANOVA. The data on disease incidence and other percentage values were subjected to arcsine square root transformation before being analyzed. The means of the treatment were compared based on Tukey's honestly significant difference (HSD) at 0.05 ($P \leq 0.05$) probability level. The statistical analysis was done using Statistical Analysis Software (SAS, 2013).

Experimental Results

Evaluation of Antifungal Activities of the PGPR Strains

A total of 158 PGPR strains were isolated from the rhizosphere soil were subjected to an *in-vitro* confrontation assay (details of the 158 strains provided in Supplementary Table 1 and Supplementary Fig. 1) and the results indicated that only 18 strains (CRM-13, CRM-18, CRM-53, CRM-57, CRM-72, CRM-75, CRM-79, CRM-80, CRM-83, CRM-98, CRM-99, CRM-105, CRM-114, CRM-121, CRM-133, CRM-149, CRM-152, CRM-156) provided more than 80% efficacy against *Rhizoctonia solani*, and 35 strains provided 50–80% pathogen inhibition (Supplementary Table 2). Fifty-two strains

that proved significant inhibition against *R. solani* were taken for characterization and other studies.

Morphological, Biochemical, and Molecular Characterization of Promising PGPR Strains

All 52 strains were given positive reactions for spore formation and negative reactions for pigmentation. They produced rod-shaped cells of small to medium size, and gram staining resulted in positive reactions. Additionally, most of the strains reacted negatively to the Methyl Red test, and urease tests. All 52 gave a positive reaction to acid production, H_2S production, and catalase tests (Supplementary Table 3). The growth of PGPR strains at different incubation temperatures was assessed. Temperature influenced the growth rate, with all 52 strains showing better growth at 28 °C, whereas none of the strains grew at 41 °C. Growth of all strains at pH 7 was significantly superior to (mean OD value 0.320) other pH levels, which was followed by pH 6 (mean OD value 0.273) while growth at pH 8 was minimum (mean OD value 0.051).

Further, we performed 16 s rDNA sequencing analysis of all 52 strains, and the sequences were checked with earlier available NCBI sequences through the blast. The 16 s rDNA of all the 52 isolates were amplified with pA and pH-specific primers. The amplified PCR product were separated in a gel electrophoresis to visualize a 1540 bp amplicon. Phylogenetic analysis of 16 s rDNA sequence analysis showed that, out of 52 strains, four strains were identified as *Bacillus subtilis*, 10 strains as *Bacillus cereus*, 10 strains were *Bacillus megaterium*, 12 strains were *Paenibacillus* spp., and three strains were *Agromyces* spp. the remaining bacterial genera were *Bacillus brevis, B. nealsonii, B. pumilus, B. toyonensis, B. velezensis, Brevibacillus borstelensis*, *Microbacterium paraoxydans, Microbacterium resistance, Ochrobacterium intermedium*, *Peribacillus simplex, Stenotrophomonas maltophilia* and *Staphylococcus pasteuri* (Supplementary Table 4). Phylogenetic analysis of all 52 strains along with 18 reference strains based on 16 s rDNA gene using neighbor joining (NJ) revealed that all the strains were grouped into two major clusters (Fig. 1). Major cluster -I divided into two subclusters 1A (having ten strains) and 1B (having twenty-six strains). Major cluster-II was again divided into subclusters II-A (30 strains) and II-B (4 strains). In Genus *Bacillus*, except for *Bacillus megaterium,* all other species (*B. subtilis, B. cereus, B. velezensis,* and other *Bacillus* species) fall under cluster-II. All Genus *Paenibacillus* fall under the major Cluster-I except *P. silvae*. The remaining species were distributed among the phylogenetic tree. The results indicated significant genetic diversity among the PGPR strains isolated from medicinal plants.

Seed Priming of Promising PGPR Strains On Growth-Promoting Activities Under *In-Vitro* Condition

Ten bacterial strains, namely CRM-13 (*Bacillus velezensis* from Garcinia), CRM-18 (*Bacillus megaterium* from *Asparagus racemosus*), CRM-72 (*Bacillus toyonensis* from Sarpagandha), CRM-105 (*Paenibacillus cucumis* from holy basil), CRM-114 (*Paenibacillus silviae* from Karanja), CRM-116 (*Paenibacillus ginsengarvi* from turmeric), CRM-133 (*B. megaterium* from rice), CRM-149 (*Bacillus subtilis* from rice), CRM-152 (*B. megaterium* from Mahanadi river bed soil), CRM-156 (*Bacillus cereus* from Mahanadi riverside) proved their efficacy with more than 80% pathogen suppression under *in-vitro* assay and only these strains were taken for further studies. Seed priming of the selected PGPR strains promoted the growth promotion of rice seedlings (Table 1, Supplementary Fig. 1). Significantly more germination (91.67% and 94.00%) was recorded in the treatment of *B. megaterium*-CRM-133 in both Tapaswini and CR-1014, respectively, whereas the treatments like *B. megaterium*-CRM-18 and *B. toyonensis*-CRM-72 were recorded with > 87% germination in the respective treatments ($P < 0.0001$) (Table 1). The lowest germination was recorded in untreated control with 64.67 and 58.33% germination in Tapaswini and CR-1014, respectively. Similarly, maximum root length was recorded in *B. toyonensis*-CRM-72 ($P < 0.0001$) in both varieties compared to other treatments. Maximum shoot length was recorded in *B. megaterium*-CRM-133 of Tapaswini and *B. toyonensis*-CRM-72 of CR-1014 ($P < 0.0001$). Maximum Vigor Index-I of 3016.33 and 3446.63 were recorded in *B. megaterium*-CRM-133 in Tapaswini and CR-1014, respectively. The maximum seedling dry weight was recorded in CRM-18, CRM-72, CRM-116 followed by CRM-105, CRM-133 in Tapaswini ($P < 0.0001$). Similarly, in CR-1014 maximum seedling dry weight was recorded in CRM-18 ($P < 0.0001$). Treatments, *B. megaterium*-CRM-133 and CRM-18 showed significant efficacy concerning for vigor index-II in Tapaswini and CR-1014, respectively, when compared to other treatments. Similarly, tiller numbers after 45 days were assessed and the results indicated that *B. megaterium*-CRM-133 recorded maximum tiller numbers (16.33 and 14.33) in both Tapaswini and CR-1014, respectively. This indicated that the tested PGPR strains can enhance seedling biomass, vigor index, number of tillers/hill, and plant height compared to untreated control (Table. 2).

The present study also assessed the efficacy of PGPR strains in reducing disease severity under controlled conditions. The results revealed that *B. toyonensis*-CRM-72 had the lowest disease incidence (57.78 and 44.19% PDI) followed by the rest of the treatments ($P < 0.0001$) in both the tested varieties. Similarly, the least relative lesion height (%)

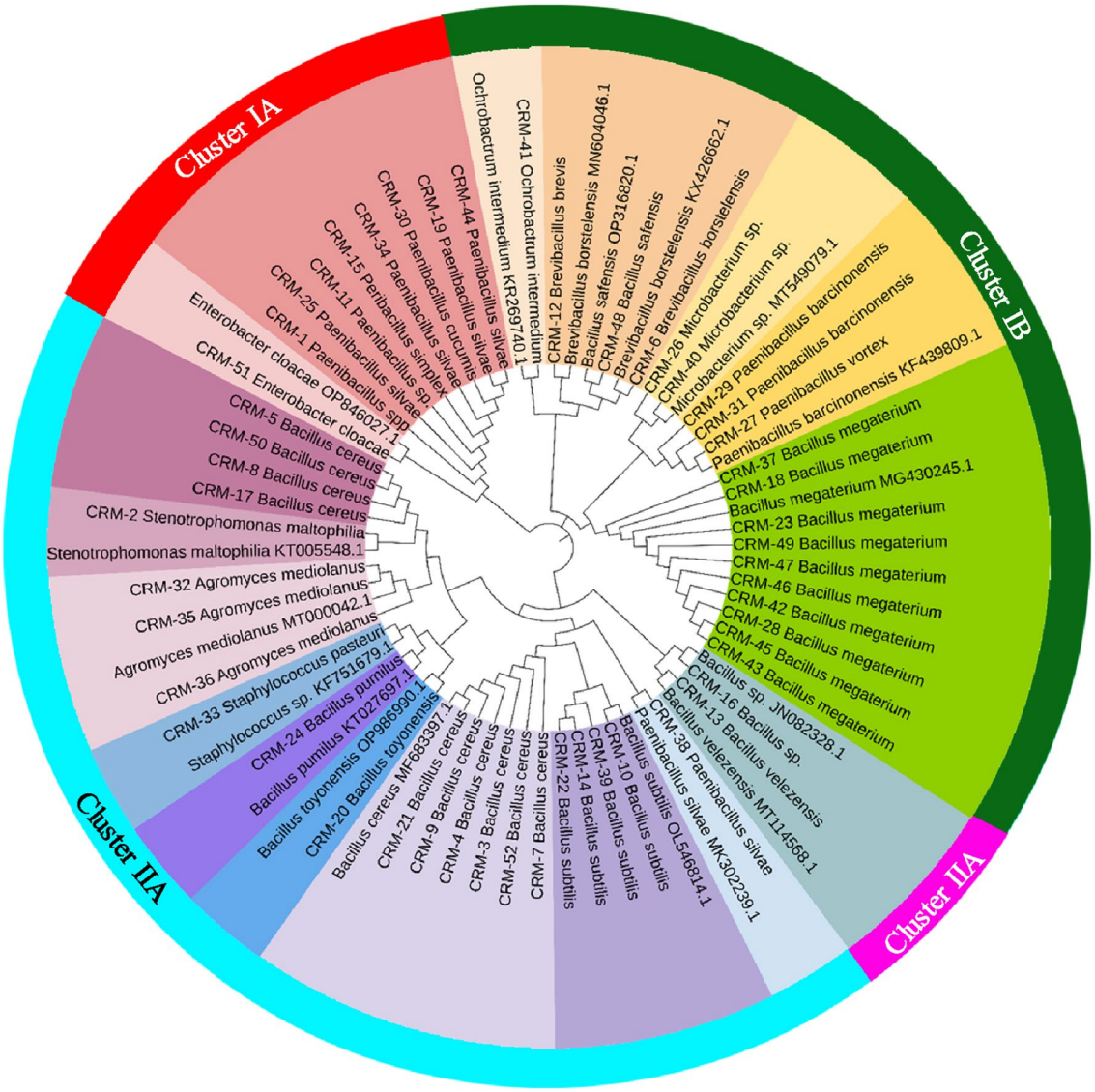

Fig. 1 Phylogenetic analysis of PGPR strains based on 16 s rDNA gene using neighbor joining (NJ) method

was recorded in *B. toyonensis*-CRM-72 (22.78 and 17.71) when compared to other treatments. We have assessed the number of days taken for sclerotia formation on inoculated plants in each treatment. The results showed that *B. toyonensis*-CRM-72 recorded maximum days (34.04–36.87 days) for sclerotia formation of *R. solani,* indicating the delayed disease spread and sclerotia formation. These results indicated that PGPR strains not only to enhance plant growth but also reduce the disease incidence significantly by reducing infection rate, reduced rate of spread (RLH), and delayed sclerotia formation, indicating disease suppression activities (Table 3).

Grain yield was recorded in all the treatments and results indicated that maximum grain yield (70.67 g and 56.00 g/pot) was recorded in *B. toyonensis*-CRM-72 in both varieties compared to the rest of the treatments. A significant increase in grain yield was also recorded from *B. megaterium*-CRM-133, *B. megaterium*-CRM-18, and *B. velezensis*-CRM-13 when compared to the untreated control. The results from pot experiments indicated that PGPR strains *B. toyonensis*-CRM-72, followed by *Bacillus megaterium*-CRM-18 and *B. velezensis*-CRM-13 not only promoted plant growth but also suppressed sheath blight incidence along with a significant increase in grain yield.

Plant Defense Enzymes Activities

All PGPR-treated plants had significantly higher activity or expression of stress-related defense enzymes compared to untreated control (Fig. 2). Maximum POD activity

Table 1 Effect of seed priming and seedling root dip of rice with PGPR strains on plant growth-promoting traits

Treatment	Treatment details	Germination (%)		Root length (cm)		Shoot length (cm)		Vigor index-I	
		Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014
T-1	*CRM-13 (Bacillus velezensis)*	86.33 (68.32)^{AB}	90.00(71.62)^{AB}	9.33(17.77)^{AB}	9.58(18.03)^{ABC}	17.33(24.60)^{B}	22.17 (28.08)^{A}	2302.25^{BC}	2857.00^{BC}
T-2	CRM-18 (*Bacillus megaterium)*	87.67 (69.50)^{AB}	87.33(69.17)^{BC}	9.25(17.70)^{AB}	11.08(19.43)^{ABC}	18.58(25.53)^{AB}	22.58 (28.36)^{A}	2436.50^{BC}	2940.25^{ABC}
T-3	CRM-72 *(Bacillus toyonensis)*	87.67 (69.50)^{AB}	90.33(71.95)^{AB}	11.42(19.74)^{A}	12.00(20.26)^{AB}	11.42(26.14)^{AB}	25.08(30.05)^{A}	2702.75^{AB}	3351.75^{AB}
T-4	CRM-105 (*Paenibacillus cucumis)*	82.67 (65.55)^{B}	81.33(64.46)^{C}	8.58(17.03)^{ABC}	9.33(17.78)^{ABCD}	16.33(23.83)^{B}	20.00(26.56)^{A}	2058.00^{C}	2382.50^{C}
T-5	CRM-114 (*Paenibacillus silviae*)	80.00 (63.45)^{B}	81.33(64.42)^{C}	8.93(17.39)^{AB}	9.42(17.86)^{ABC}	17.67(24.85)^{B}	20.57(26.96)^{A}	2127.03^{C}	2440.63^{C}
T-6	CRM-116 (*Paenibacillus ginsengarvi*)	82.33 (65.18)^{B}	84.33(66.71)^{BC}	8.70(17.14)^{ABC}	9.33(17.78)^{ABCD}	17.25(24.53)^{B}	20.92(27.21)^{A}	2133.98^{C}	2549.42^{C}
T-7	CRM-133 (*Bacillus megaterium*)	91.67 (73.34)^{A}	94.00(75.85)^{A}	10.33(18.75)^{AB}	12.33(20.55)^{A}	22.58(28.36)^{A}	24.35(29.56)^{A}	3016.33^{A}	3446.63^{A}
T-8	CRM-149 (*Bacillus subtilis*)	86.33 (68.32)^{AB}	86.33(68.32)^{BC}	9.25(17.69)^{AB}	8.58(17.01)^{BCD}	17.50(24.72)^{B}	20.58(26.97)^{A}	2311.17^{BC}	2518.33^{C}
T-9	CRM-152 (*Bacillus megaterium*)	83.67 (66.18)^{B}	85.00(67.22)^{BC}	9.08(17.53)^{AB}	8.76(17.18)^{BCD}	8.76(24.47)^{B}	24.25(29.49)^{A}	2196.17^{C}	2805.41^{BC}
T-10	CRM-156 *(Bacillus cereus)*	79.67 (63.22)^{B}	84.33(66.71)^{BC}	8.42(16.86)^{BC}	8.33(16.77)^{CD}	17.92(25.03)^{B}	21.17(27.38)^{A}	2099.00^{C}	2490.83^{C}
T-11	Fungicide control	85.00 (67.28)^{AB}	86.00(68.05)^{BC}	7.58(15.97)^{BC}	8.33(16.77)^{CD}	18.42(25.40)^{AB}	22.42(28.26)^{A}	2209.16^{C}	2645.50^{C}
T-12	Untreated check	64.67 (53.54)^{C}	58.33(49.81)^{D}	6.25(14.46)^{C}	6.30(14.51)^{D}	11.00(19.35)^{C}	12.17(20.39)^{B}	1118.42^{D}	1081.25^{D}
p-Value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV (%)		2.75	2.36	4.55	5.22	3.77	3.63	5.67	6.05
SE(d)		1.48	1.29	0.6	0.76	0.761	0.81	103.11	129.79
Tukey HSD at 5%		6.48	5.65	2.81	3.32	3.32	3.56	451.01	567.75

The data presented in the parenthesis are the corresponding arcsine square root transformed values. Values with the same English letter within the same column do not significantly differ from each other according to Tukey's HSD test at $P < 0.05$

was recorded in *B. toyonensis*-CRM-72 (2.23 and 2.35 δ absorbance/min/mg protein), followed by *B. megaterium*-CRM-18 and *B. velezensis*-CRM-13 in both Tapaswini and CR-1014. Similarly, significantly superior SOD activity was recorded in *B. toyonensis*-CRM-72 (12.67 and 13.58 unit/min/g of tissue), followed by *B. megaterium*-CRM-18 and *B. velezensis*-CRM-13. Similarly, the maximum CAT activity was recorded in *B. toyonensis*-CRM-72 (35.17 and 36.25Unit activity/Min/g of FW), followed by *B. megaterium*-CRM-18 and *B. velezensis*-CRM-13. All the other treatments recorded significantly higher activity of defense-related enzymes compared to untreated control. Principal component analysis also conformity with our results as it has grouped the enzyme expression of the *B. toyonensis*-CRM-72, *B. megaterium*-CRM-18, and *B. velezensis*-CRM-13 in the same compartment for both varieties and control group placed in a separate compartment (Supplementary Fig. 3). This indicates that PGPR has a definite role in induced resistance in rice against *R. solani*.

Table 2 Effect of seed priming and seedling root dip of rice with PGPR strains on plant growth-promoting traits

Treatment	Treatment details	Seedling dry weight		Vigor index-II		Number of tillers/hill after 45 DAT		Plant height after 45 DAT	
		Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014
T-1	*CRM-13 (Bacillus velezensis)*	0.16(2.31)^{A}	0.17(2.36)^{BC}	14.10(22.04)^{AB}	15.30(23.01)^{BCD}	13.00(21.12)^{ABC}	11.33(19.65)^{ABC}	42.67(40.78)^{ABC}	54.33(47.49)^{A}
T-2	CRM-18 (*Bacillus megaterium)*	0.19(2.50)^{A}	0.23(2.77)^{A}	16.66(24.08)^{A}	20.38(26.82)^{A}	14.00(21.96)^{ABC}	13.67(21.69)^{AB}	47.67(43.66)^{A}	56.33(48.64)^{A}
T-3	CRM-72 *(Bacillus toyonensis)*	0.19(2.52)^{A}	0.17(2.56)^{AB}	16.95(24.30)^{A}	18.07(25.14)^{ABC}	15.00(22.78)^{AB}	14.33(22.21)^{A}	45.67(42.51)^{AB}	55.33(48.06)^{A}
T-4	CRM-105 (*Paenibacillus cucumis)*	0.17(2.31)^{A}	0.14(2.36)^{BC}	13.50(21.53)^{AB}	13.83(21.81)^{CD}	13.00(21.12)^{ABC}	10.00(18.72)^{BC}	41.00(39.81)^{ABC}	50.33(45.19)^{AB}
T-5	CRM-114 (*Paenibacillus silviae*)	0.15(2.36)^{A}	0.16(2.17)^{CD}	13.60(21.64)^{AB}	16.66(19.95)^{D}	13.00(21.12)^{ABC}	10.00(18.38)^{C}	40.33(39.42)^{BC}	47.33(43.47)^{B}
T-6	CRM-116 (*Paenibacillus ginsengarvi*)	0.19(2.22)^{A}	0.21(2.31)^{BC}	12.35(20.56)^{B}	13.77(21.76)^{CD}	13.67(21.68)^{ABC}	10.00(18.42)^{C}	40.67(39.62)^{ABC}	45.00(42.13)^{B}
T-7	CRM-133 (*Bacillus megaterium*)	0.17(2.47)^{A}	0.18(2.60)^{AB}	17.11(24.43)^{A}	19.43(26.14)^{AB}	16.33(23.84)^{A}	14.33(22.21)^{A}	45.00(42.13)^{AB}	56.33(48.)
T-8	CRM-149 (*Bacillus subtilis*)	0.15(2.34)^{A}	0.17(2.41)^{ABC}	14.39(22.29)^{AB}	15.25(22.97)^{BCD}	13.00(21.12)^{ABC}	12.33(20.54)^{ABC}	39.67(39.03)^{BC}	50.33(45.19)^{AB}
T-9	CRM-152 (*Bacillus megaterium*)	0.16(2.22)^{A}	0.16(2.34)^{BC}	12.55(20.74)^{B}	14.17(22.80)^{CD}	13.00(21.12)^{ABC}	13.67(21.68)^{AB}	40.33(39.42)^{BC}	48.00(43.85)^{B}
T-10	CRM-156 *(Bacillus cereus)*	0.17(2.32)^{A}	0.19(2.31)^{BC}	13.01(21.14)^{B}	13.77(21.78^{CD}	11.00(19.36)^{C}	11.00(19.36)^{ABC}	37.67(37.86)^{C}	46.33(42.90)^{B}
T-11	Fungicide control	0.10(2.36)^{A}	0.10(2.47)^{ABC}	6.47(22.34)^{AB}	16.06(23.61)^{ABC}	12.00(20.26)^{BC}	11.67(19.97)^{ABC}	41.67(40.20)^{ABC}	50.33(45.19)^{AB}
T-12	Untreated check	0.16(1.81)^{B}	0.17(1.84)^{D}	14.69^{C}	6.03(14.16)^{E}	6.33(14.51)^{D}	6.67(14.90)^{D}	27.67(31.72)^{D}	35.00(36.27)^{C}
p-Value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV(%)		3.88	4.45	3.79	4.52	4.51	4.51	2.94	2.22
SE(d)		0.073	0.086	0.670	0.828	0.767	0.730	0.952	0.809
Tukey HSD at 5%		0.3202	0.3778	2.931	3.6196	3.3532	3.1925	4.1633	3.5406

The data presented in the parenthesis are the corresponding arcsine square root transformed values. Values with the same English letter within the same column do not significantly differ from each other according to Tukey's HSD test at $P < 0.05$

Seed Priming with Promising PGPR Strains On Plant Growth and Disease Reduction

The promising strains were further tested under field conditions and the results (Table 4) indicated that the significantly lowest disease incidence was recorded in *B. toyonensis*-CRM-72 ($P < 0.0001$) with 17.37 and 12.88% disease incidence in both Tapaswini and CR-1014 followed by *B. megaterium*-CRM-18 and *B. velezensis*-CRM-13. Maximum disease incidence was recorded in the untreated control (85.00 and 23.54%) after 30 days.

Similarly, *B. toyonensis*-CRM-72 treatment recorded the least relative lesion height (%) compared to other treatments. In Tapaswini, *B. toyonensis*-CRM-72 treatment recorded 79.56% disease control efficacy over the untreated check. In CR-1014, we recorded 84.85% disease reduction over control (data not provided). We also recorded least the AUDPC (area under disease progress curve) values in *B. toyonensis*-CRM-72 treatment in both the tested varieties (382.98 and 286.25) indicating disease-suppressive activities. All the other PGPR strains significantly reduced disease incidence, relative lesion height, and AUDPC values when compared to untreated control. The maximum number of effective tillers/mt2, panicle length, panicle weight, 1000 grain weight, and number of filled grains were recorded in T-3 followed by T-2 and T-1 ($P < 0.0001$). Final grain yield was recorded

Table 3 Effect of seed priming and seedling root dip of rice with PGPR strains on control of sheath blight disease under pot conditions

Treatment	Treatment details	Disease incidence (%)		Relative lesion height (%)		Sclerotia formation (Days)		Grain yield/pot (gm)	
		Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014
T-1	*CRM-13 (Bacillus velezensis)*	71.79(57.82)^{B}	61.76(51.78)^{AB}	31.91 (34.40)^{CDE}	27.98 (31.96)^{BCDE}	26.00 (30.65)^{ABC}	28.33 (32.16)^{BC}	60.33 (50.97)^{B}	45.00 (42.13)^{BC}
T-2	CRM-18 (*Bacillus megaterium)*	64.29 (53.38)^{B}	46.34 (43.09)^{B}	26.99 (31.27)^{DE}	20.85 (27.18)^{DE}	28.67 (32.35)^{AB}	33.67 (35.46)^{AB}	62.00 (51.95)^{B}	48.00 (43.85)^{AB}
T-3	CRM-72 *(Bacillus toyonensis)*	57.78(49.81)^{B}	44.19 (41.63)^{B}	22.78 (28.48)^{E}	17.71 (24.91)^{E}	31.33 (34.04)^{A}	36.00 (36.85)^{A}	70.67(57.21)^{A}	56.00 (48.45)^{A}
T-4	CRM-105 (*Paenibacillus cucumis)*	64.10 (53.21)^{B}	80.65(70.11)^{AB}	41.06 (39.85)^{BCD}	38.47 (38.33)^{BC}	17.00 (24.34)^{EF}	19.67 (26.31)^{DE}	49.00 (44.43)^{DE}	35.33 (36.46)^{DE}
T-5	CRM-114 (*Paenibacillus silviae*)	69.23 (56.45)^{B}	83.33(66.95)^{AB}	49.28 (44.60)^{B}	40.47 (39.52)^{B}	16.67 (24.08)^{EF}	16.67 (24.08)^{E}	42.33 (40.59)^{F}	25.67 (30.43)^{F}
T-6	CRM-116 (*Paenibacillus ginsengarvi*	65.85 (54.61)^{B}	80.00(68.44)^{AB}	44.79 (42.05)^{BC}	39.12 (38.75)^{BC}	14.50 (22.38)^{F}	19.00 (25.84)^{DE}	45.33 (42.32)^{EF}	29.00 (32.58)^{EF}
T-7	CRM-133 (*Bacillus megaterium*)	69.39 (56.71)^{B}	69.77 (57.56)^{AB}	36.05 (36.89)^{BCDE}	26.29 (30.85)^{CDE}	23.50 (28.99)^{BCD}	28.00 (31.95)^{BC}	57.00 (49.02)^{BC}	33.33 (35.26)^{DE}
T-8	CRM-149 (*Bacillus subtilis*)	87.18 (69.31)^{AB}	72.97 (59.24)^{AB}	37.13 (37.57)^{BCDE}	29.49 (32.90)^{BCDE}	21.00 (27.27)^{CDE}	27.00 (31.30)^{BC}	56.33 (48.64)^{BC}	39.00 (38.64)^{CD}
T-9	CRM-152 (*Bacillus megaterium*)	71.79 (57.90)^{B}	73.17 (59.05)^{AB}	39.39 (38.87)^{BCD}	33.70 (35.50)^{BCD}	20.00 (26.55)^{DE}	25.00(30.00)^{CD}	52.67 (46.53)^{CD}	38.00 (38.25)^{CD}
T-10	CRM-156 *(Bacillus cereus)*	84.85 (67.41)^{AB}	75.76 (61.89)^{AB}	41.84 (40.31)^{BCD}	34.52 (36.04)^{BC}	20.67 (27.03)^{CDE}	23.67 (29.10)^{CD}	51.33 (45.76)^{CDE}	36.33 (37.07)^{DE}
T-11	Fungicide control	72.73 (64.70)^{AB}	65.63 (54.67)^{AB}	38.23 (38.18)^{BCD}	32.83 (35.00)^{BCD}	27.00 (31.30)^{AB}	29.33 (32.79)^{ABC}	56.67 (48.83)^{BC}	45.00 (42.13)^{BC}
T-12	Untreated check	94.74 (83.10)^{A}	95.00 (82.60)^{A}	82.12 (66.06)^{A}	63.78 (53.22)^{A}	7.00 (15.32)^{G}	15.67 (23.29)^{E}	24.00 (29.33)^{G}	17.67 (24.82)^{G}
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV (%)		10.05	18.12	6.57	6.69	4.02	4.11	2.30	3.51
SE(d)		4.952	8.839	2.139	1.930	0.888	1.004	0.870	1.075
Tukey HSD at 5%		21.662	38.663	9.3541	8.4402	3.8828	4.3909	3.8056	4.7008

The data presented in the parenthesis are the corresponding arcsine square root transformed values. Values with the same English letter within the same column do not significantly differ from each other according to Tukey's HSD test at $P < 0.05$

in all the treatments and results indicated that maximum grain yield (63.33q/h and 48.00q/h) was recorded in T-3 followed by T-2 and T-1 in both the varieties ($P < 0.0001$). The untreated control recorded the lowest grain yield (14.00 and 13.00 q/h), maximum disease incidence, and AUDPC values. From the above results, it is clear that the PGPR strains isolated from medicinal plants and the rice rhizosphere not only promoted plant growth but also suppressed sheath blight disease.

Discussion

Rice being an imperative food crop suffers from many diseases. For the management of rice diseases, integrated management practices include using resistant varieties, biological control agents, and chemical management. Though chemical management displays quick, intensive, and effective results, it also has several disadvantages. On the other hand, biological control can provide a safer and more eco-friendly treatment strategy. Antagonistic microorganisms isolated from a variety of sources, including the rhizosphere, phyllosphere, rhizoplane, and phylloplane,

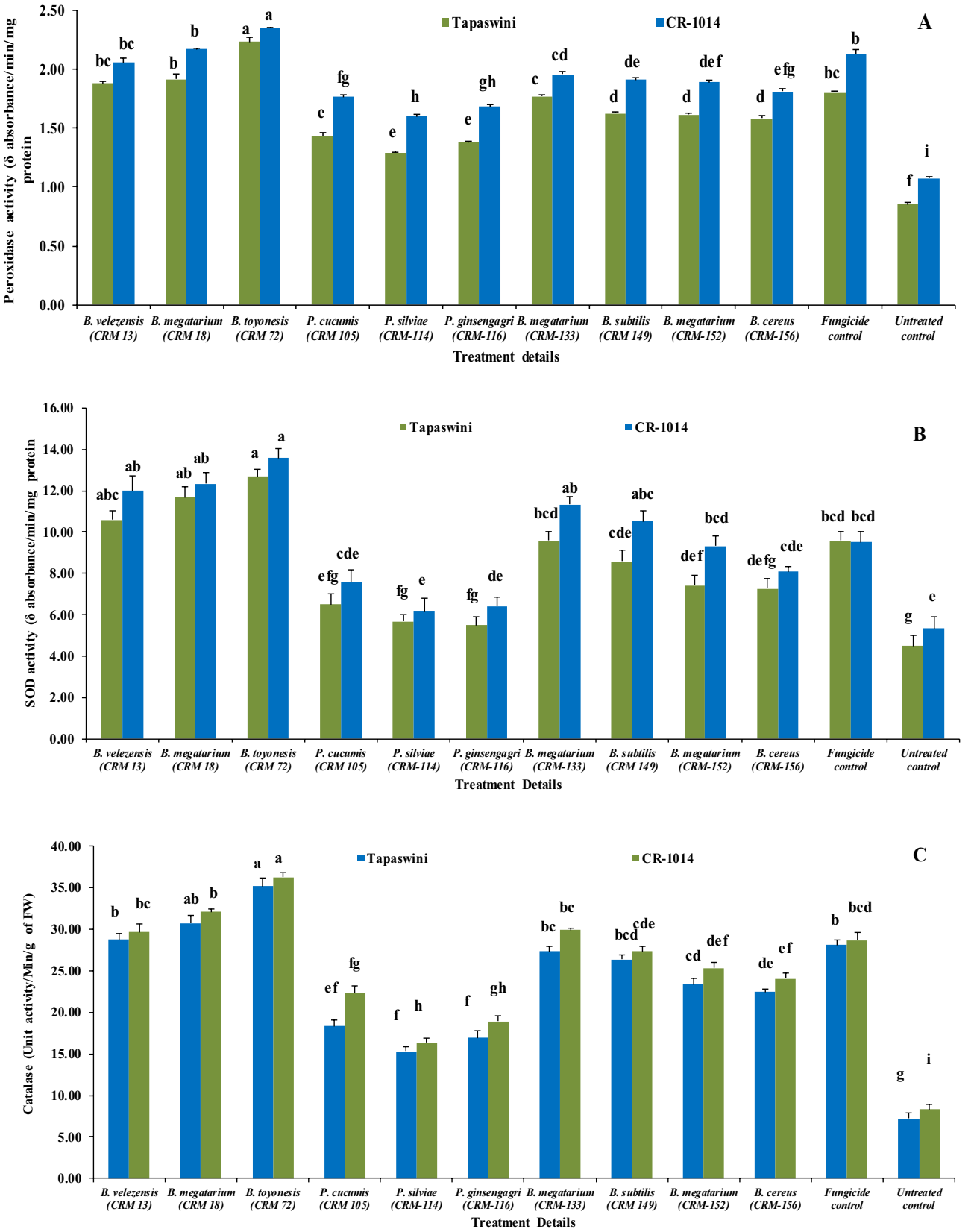

Fig. 2 Effect of plant growth-promoting rhizobacteria (PGPR) and fungicide treatments on **A** Peroxidase, **B** superoxide dismutase, and **C** catalase (CAT) activities in rice varieties. Different letters in each strain of a particular enzyme denote significant difference at $P < 0.05$ according to a Tukey test

Table 4 Effect of seed priming and seedling root dip of rice with PGPR strains on control of sheath blight disease under field conditions

Treatment	Treatment details	Disease incidence after 30 days of inoculation (%)		Relative lesion height (%) after 30 days of inoculation		AUDPC		Grain yield/ha	
		Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014	Tapaswini	CR-1014
T-1	*CRM-13 (Bacillus velezensis)*	27.10 (31.37)^{F}	17.83(24.97)^{GH}	32.21(34.58)^{FG}	16.71 (24.12)^{FG}	603.63	388.63	52.41 (46.39)^{BC}	42.42 (40.64)^{BC}
T-2	CRM-18 (*Bacillus megaterium)*	18.36 (25.36)^{G}	15.83(23.44)^{H}	29.21 (32.71)^{GH}	15.67 (23.31)^{GH}	411.43	351.25	54.17 (47.39)^{B}	45.25 (42.27)^{AB}
T-3	CRM-72 *(Bacillus toyonensis)*	17.37 (24.62)^{G}	12.88 (21.00)^{I}	27.71 (31.76)^{H}	12.88 (21.02)^{H}	382.98	286.25	63.33 (52.74)^{A}	48.00 (43.85)^{A}
T-4	CRM-105 (*Paenibacillus cucumis)*	34.79 (36.14)^{CD}	25.92 (30.60)^{C}	42.63 (40.76)^{C}	26.92 (31.25)^{C}	774.38	563.13	40.48(39.51)^{EFG}	33.00 (35.06)^{EFG}
T-5	CRM-114 (*Paenibacillus silviae*)	42.67 (40.78)^{B}	31.58 (34.19)^{B}	46.08 (42.75)^{B}	31.60 (34.20)^{B}	957.50	685.63	34.83 (36.16)^{G}	29.00 (32.57)^{G}
T-6	CRM-116 (*Paenibacillus ginsengarvi*)	38.37 (38.27)^{C}	25.25(30.16)^{CD}	43.21 (41.10)^{BC}	28.92 (32.53)^{BC}	853.63	568.13	38.75 (38.50)^{FG}	30.50 (33.52)^{FG}
T-7	CRM-133 (*Bacillus megaterium*)	27.67 (31.73)^{EF}	19.21(25.99)^{FG}	33.83 (35.56)^{F}	19.17 (25.95)^{EF}	613.13	425.00	46.00(42.70)^{CDE}	40.41 (39.47)^{BCD}
T-8	CRM-149 (*Bacillus subtilis*)	29.29 (32.77)^{EF}	20.96(27.24)^{EF}	34.88 (36.20)^{EF}	20.17 (27.06)^{E}	651.88	461.88	44.08(41.60)^{DEF}	39.43 (38.90)^{CD}
T-9	CRM-152 (*Bacillus megaterium*)	31.00 (33.83)^{DE}	22.49(28.31)^{DE}	38.00 (38.05)^{DE}	22.33 (28.20)^{DE}	690.63	487.93	43.92(41.50)^{DEF}	36.33 (37.07)^{DE}
T-10	CRM-156 *(Bacillus cereus)*	33.46 (35.33)^{D}	23.88(29.25)^{CDE}	40.38 (39.45)^{CD}	25.79 (30.52)^{CD}	744.38	526.88	41.00(39.81)^{EFG}	35.67 (36.67)^{DEF}
T-11	Fungicide control	26.13 (30.72)^{F}	19.04(25.87)^{FG}	34.00 (35.67)^{F}	19.08 (25.89)^{EF}	1890.00	867.50	48.33(44.04)^{BCD}	39.17 (38.74)^{CD}
T-12	Untreated check	85.00 (67.22)^{A}	39.54(38.96)^{A}	71.92 (58.00)^{A}	37.63 (37.83)^{A}	603.63	388.63	14.00 (21.95)^{H}	13.00 (21.12)^{H}
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001			< 0.0001	< 0.0001
CV(%)		2.26	2.42	1.66	2.83			3.17	2.91
SE(d)		0.659	0.560	0.527	0.659			1.063	0.870
Tukey HSD at 5%		2.3959	2.0357	1.9154	2.3987			3.8659	3.1635

The data presented in the parenthesis is the corresponding arcsine square root transformed values. Values with the same English letter within the same column do not significantly differ from each other according to Tukey's HSD test at $P < 0.05$

play an important role in soil microbial equilibrium and are potent allies against a variety of biotic and abiotic stresses (Ana et al. 2009). Most microbes can promote plant growth and development. In addition, they are also involved in various complex physiological processes in the plant system (Gavriilidou et al. 2022). Thus, microbes

can fix nutrients essential for plant growth, like nitrogen, decompose organic matter, and improve soil composition and structure (Jacoby et al. 2017). Most importantly, they have multiple modes of action that benefit plants against harmful pests, pathogens, and other abiotic stresses (Goswami et al. 2016; Hashem et al. 2019). In the present investigation, 158 strains were obtained from the rhizosphere soil of medicinal plants, rice, and Mahanadi River bed soil were evaluated against the major fungal pathogen of rice, i.e., *Rhizoctonia solani*. Based on their antifungal properties, 52 effective strains were selected for characterization using morphological and molecular methods. We identified four strains as *Bacillus subtilis*, 10 strains as *Bacillus cereus*, 10 strains were *Bacillus megaterium*, 11 strains were *Paenibacillus* spp., and three strains were *Agromyces* spp. the remaining isolates were *B. brevis, B. nealsonii, B. pumilus, B. toyonensis, B. velezensis, Brevibacillus borstelensis*, *Microbacterium paraoxydans, M. resistance, Ochrobacterium intermedium*, *Peribacillus simplex, Staphylococcus pasteuri,* and *Stenotrophomonas maltophilia*. Rhizosphere soil bacteria like *Bacillus, Burkholderia, Enterobacter, Pseudomonas, Serratia, Arthrobacter, Achromobacter, Micrococcus, Flavobacterium, Azospirillum, Azotobacter* were reported to have antifungal and wide range of PGP activities (Vessey 2003; Forchetti et al. 2007; Felici et al. 2008; Swain and Ray 2009; Sandilya et al. 2022).

With the intention of utilizing biological control agents for disease management, the present study examined the 10 most effective Plant growth-promoting rhizobacteria (PGPR) strains for their plant growth-promoting effects and disease-suppressive activities. Among the selected strains, *Bacillus toyonensis (CRM-72)* isolated from *Rauvolfia serpentine*, *Bacillus velezensis (CRM-13)* isolated from *Garcinia cowa, Bacillus megaterium (*CRM-18) from *Asparagus racemosus*, and other strains belonging to *Bacillus subtilis, Bacillus cereus,* and *Paenibacillus* proved their efficacy on plant growth promotion and induced disease resistance against sheath blight. Several reports suggest that the plant growth promotion and disease-suppressive activities of these rhizosphere soil bacteria may be due to the multiple actions of PGPR in beneficial effects (Antoun et al. 2005; Bashan and De-Bashan 2010; Kashyap et al. 2021). Similar to our results, *Bacillus* strains recovered from rhizospheric soils of the Indo Gangetic plains strongly inhibited the growth of *Rhizoctonia solani* and *Fusariun oxysporum* under in-vitro condition (Devi et al. 2023). Plant growth promotion may be enhanced due to the induction of systemic resistance, antibiosis, competitive omission, and other mechanisms (Tripathi et al. 2012). A large number of bacterial with multipronged activities are exploited from different sources and commercially utilized in the eco-friendly management of stress (Antoun et al. 2005; Bashan and De-Bashan 2010; Rodríguez-Díaz et al. 2008; Tripathi et al. 2012; Kaul et al. 2022).

In the present investigation, an attempt has been made to exploit the antifungal activity of bacterial strains through dual-culture antimicrobial assay. Most species from *Bacillus* and *Paenibacillus* showed potential antagonistic activity against *R. solani*. This was further confirmed by screening antibiotic-producing genes using gene-specific markers. Interestingly, all the tested isolates could amplify the Fengycine (*fenb*), Iturin (*ituD*), and Surfactin (*srfA*) genes, which are involved in antibiotic biosynthesis. Previous studies also identified the antibiotics such as kanosamine, oligomycin A, xanthobaccin, and zwittermicin in *Streptomyces*, *Bacillus*, and *Stenotrophomonas* spp. (Abbas et al. 2018).

The promising bacterial strains with antifungal activity were further assessed for plant growth parameters through seed biopriming. The percent seed germination and agronomical parameters like plant height, root length including plant biomass production were significantly higher when treated with *Bacillus toyonensis* (CRM-72 to un-inoculated control and other treatments). This altered phenotype with enhanced growth of rice plants in response to the seed priming and seedling dip with PGPRs was almost similar to the observations of Raj et al. 2004, who reported that seed biopriming mediated crop seedling growth and enhanced resistance. Therefore, seed treatment with PGPR can be significantly beneficial for plant growth and development (Samreen et al. 2021).

Plant cell consists of specialized antioxidative defense mechanisms involving enzymes such as Peroxidase (POD), Superoxide dismutase (SOD), Catalase, and Ascorbate peroxidase. These enzymes play a key role in the scavenging and detoxification of reactive oxygen species (ROS) and prevent oxidative damage to plant cells. The association of PGPR contributes to induced resistance in plants through the biosynthesis of PR proteins and other defense-related molecules, which in turn protects plants under stress conditions (Fujita and Hasanuzzaman 2022). In this study, we assess the status of Peroxidase (POD), Superoxide dismutase (SOD), and Catalase (CAT) in the PGPR-inoculated rice plants. A significant increase in the level of POD, SOD, and CAT activity was recorded in treatment T-3 followed by treatments T-2 and T-1 compared to the untreated mock and other treatments. Therefore, the results indicated that enhanced activity of antioxidative defense enzymes indeed protects plants from sheath blight disease through induced disease resistance. Bhattacharyya (2020) previously reported that rhizobacterial treatment of rice plants with *Bacillus* and other strains induces enzymatic antioxidative defense reactions against sheath blight disease. In general, PGPRs impart systemic resistance through Jasmonic acid (JA)/ethylene (ETH) and auxin pathways. Due to their higher levels of endogenous salicylic acid (SA) in rice, the SA-dependent

pathways are the preferred ways of inducing resistance within rice plants against plant pathogens. Various types of determinants have been implicated to play an important role to induce systemic resistance (ISR). They work either individually or in combination to induced plant ISRs in response to invading pathogens (Zhu et al. 2022).

Furthermore, the experiment was extended to field conditions where all the 10 strains and controls were evaluated with replications. The results were promising as all the PGPR strains significantly increased effective tillers/mt^2, panicle length, number of filled grains, and grain weight. They also significantly suppressed the sheath blight disease incidence by less PDI, least relative lesion height (%), and AUDPC. Apart from decreasing disease incidence, they also enhanced grain yield significantly. Out of 10 PGPR strains evaluated *Bacillus toyonensis (CRM-72)* isolated from *Rauvolfia serpentina*, *Bacillus velezensis (CRM-13)* isolated from *Garcinia cowa,* and *Bacillus megaterium (CRM-18)* from *Asparagus racemosus*, outperformed on plant growth promotion and induced disease resistance against sheath blight. Several reports from the previous studies supported our results. Perello et al. (2002) reported that *B. megaterium* (strain MKB-135) not only enhanced wheat growth but also reduced septoria leaf blight (STB). Similar results were observed with *Septoria tritici* and *Puccinia recondite* disease of wheat was controlled by antibiotic-producing fluorescent pseudomonad (Levy et al. 1988, 1989; Flaishman et al. 1996; Nolan and Cook 2000; Perellò et al. 2006). Likewise, in a recent study, Kaul et al. (2022) discovered that the endophytic bacilli strain isolated from wheat could protect the plants from head scab disease and improve the plant growth by displaying plant growth-promoting traits. Interestingly, a halotolerant *Bacillus* sp. involved with destruction of different fungal pathogens associated with chickpeas (Sharma et al. 2019). Recently, this potential has been realized by Wiwattanapatapee et al. (2007) who developed effervescent fast-disintegrating granules containing endospores of *B. megaterium* as a possible biocontrol agent of *Rhizoctonia solani* Kühn, which causes leaf sheath blight of rice (*Oryzae sativa* L.). Similarly, *Bacillus velezensis* is an environmentally friendly bacterium with multiple biological functions including promoting plant growth (Meng et al. 2016), suppressing plant diseases (Nam et al. 2009), and degrading pollutants (Bafana et al. 2008). *Bacillus velezensis* has also demonstrated a potential role as a biological agent for controlling potato scab disease (Cui et al. 2020) and web blight of cowpea (Siva et al. 2023). A promising antagonistic strain for use as an effective biocontrol agent usually has broad-spectrum antimicrobial activities (Yu et al. 2017; Sun et al. 2017; Zhang et al. 2017; Rojas-Solís et al. 2018; Contreras-Pérez et al. 2019; Jiménez et al.2013). *Bacillus* spp. with PGP activities are providing a huge benefit for agriculture and plant protection. They are recognized as 'safe' bacteria that produce useful substances in crop production and industrial applications (Stein 2005;). They produce endospores that help to survive harsh environmental conditions, germination is triggered by different environmental cues, they remain viable as long-term storage of commercial products, and can also reduce the complexity of formulation processes (Collins and Jacobson 2003). Moreover, *Bacillus* species can function as plant endophytes, upon colonizing the plant interiors, plants exhibit defense pathways and protects from phytopathogens (McSpadden-Gardener and Driks 2004; Romero et al. 2004). These genera can also produce antimicrobial metabolites which can be used for substituting synthetic chemicals or they can be used as bio-pesticides and biofertilizers (Ongena et al. 2005). Overall, the present study provides identification and guidance for utilizing multi-trait PGPR strains to manage rice diseases to achieve sustainable plant protection.

Conclusion

Eco-friendly agriculture system is attracting more and more farmers as it is residue free and environmentally safe. The present study identified the effective PGPR strains *Bacillus toyonensis* and *B. megaterium* and validated this strain's efficacy against rice sheath blight disease. These strains may help to enhance the rice yield through multiple activities like plant growth promotion, nutrient cycling, and induced resistance against sheath blight fungi in the rice ecosystem. Further work on simple mass multiplication and suitable formulation with enhanced shelf life will be addressed to mitigate the sheath blight problem in rice.

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Declarations

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