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RESEARCH ARTICLE

Molecular characterization and biocontrol potential of native rhizobacteria against *Rhizoctonia solani* in rice

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Abstract

Sheath blight of rice, caused by *Rhizoctonia solani* Kuhn, ranks as the second most devastating fungal disease of rice after blast, leading to significant yield and quality losses. Current management strategies rely heavily on chemical fungicides, which pose serious environmental and health risks. In recent years, the use of native microbial antagonists has emerged as a promising, eco-friendly alternative for sustainable disease management. In the present study, 32 native rhizobacterial isolates comprising 30 actinobacteria and 2 fluorescent bacteria were obtained from the rhizosphere of healthy rice plants across major rice-growing regions of Karnataka. These isolates were evaluated for their antagonistic potential against a virulent *R. solani* isolate (RS4) under both *in vitro* and *in vivo* conditions. Among the isolates, the actinobacterial strains GVTAM 8, DWRAM 10 and the reference strain AUDT 502 exhibited significant inhibitory effects of 90.61 %, 88.38 % and 87.77 %, respectively, under *in vitro* conditions. Subsequent glasshouse experiments concluded that seed treatment followed by foliar spraying with GVTAM 8 and AUDT 502 was most effective in reducing sheath blight disease severity, recording lowest relative lesion height of 14.52 %, which was statistically on par with hexaconazole treatment. Molecular identification confirmed GVTAM 8 and DWRAM 10 as *Streptomyces cinnabarinus* and *Streptomyces pseudogriseolus*, respectively. The biocontrol activity of these actinobacteria makes them a suitable candidate for inclusion in disease management programs, thereby avoiding the complete dependency on chemicals for the management of sheath blight disease.

Keywords: actinobacteria; eco-friendly disease management; rhizobacteria, *Rhizoctonia solani*; sheath blight of rice; *Streptomyces*

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide, serving as a primary source of calories for approximately 60 % of the worlds's population (1). Ensuring stable rice production is therefore crucial for global food and nutritional security. However, rice productivity is severely constrained by multiple factors, including the shrinking availability of arable land, growing concerns over sustainable agricultural practices and most importantly, biotic and abiotic stresses (2). Among these biotic constraints, fungal diseases alone account for an annual yield loss of about 30 % (3).

Sheath blight (ShB) of rice, caused by the soil-borne necrotrophic fungus *Rhizoctonia solani* Kühn, is a highly destructive disease, ranking second only to rice blast in terms of economic impact. ShB not only causes yield losses ranging from 6 % to 70 % depending on environmental conditions, crop management and varietal susceptibility, but also adversely affects grain quality and market value (4). The increased prevalence of ShB is closely associated with the large-scale monoculturing of semi-dwarf, high-yielding varieties, excessive nitrogen fertilization, dense planting systems and changing climatic conditions that favour pathogen proliferation.

Effective management of ShB remains a significant challenge as a result of pathogen's ability to persist in soil and plant debris as sclerotia for several years, wide host range and the absence of major resistance genes in the primary gene pool. Although integrated approaches involving various cultural and chemical strategies are available, these methods are often unsustainable due to high input costs, risk the emergence of resistant pathogen strains and their negative impact on beneficial soil microorganisms, environmental health and food safety. Furthermore, the polygenic and quantitative nature of resistance in rice genotypes makes breeding for durable ShB resistance slow and complex (5).

In this context, there is an urgent need to develop eco-friendly and sustainable alternatives for ShB management. Plant growth-promoting rhizobacteria (PGPR) have emerged as a promising candidate due to their ability to effectively colonize the rhizosphere and promote plant growth simultaneously (6). Unlike chemical fungicides, PGPR-based biocontrol is environmentally benign, cost-effective and compatible with

integrated disease management (IDM) strategies. Biological control can be achieved either by introducing foreign antagonists into new habitats or, more sustainably, by promoting the proliferation of native antagonists to outcompete pathogens. The mechanism of suppression largely relies on rhizosphere competition, antibiosis, lytic enzyme production and the induction of host plant defences, thereby creating a protective buffer zone that restricts pathogen spread (7).

Despite these advantages, research on successful PGPR application against *R. solani* in rice remains limited, with most studies confined to screenings under *in vitro* conditions, with field efficacy largely unproven (8). Since *R. solani* persists in the soil for a long time and typically initiates infection at the base of seedlings near the water line and progresses upward through the foliage, forming elongated lesions, the identification of robust PGPR strains from the rice soil ecosystem with strong rhizosphere competence, adaptability and consistent performance under glasshouse and field conditions is critical. Therefore, the present study was undertaken to isolate, identify and characterize potent native rhizobacterial antagonists from the rice ecosystem and to evaluate their antagonistic potential against the ShB pathogen through systematic *in vitro* and glasshouse screening.

Materials and Methods

Soil sampling

Representative soil samples were collected from rhizosphere of healthy rice plants from major rice grown areas of Karnataka *viz*., Mugad (Dharwad) (15.15° N, 74.70° E.), Durga camp (Gangavati) (15.78° N, 76.77° E), Hebbala (Gangavati) (15.55° N, 76.40° E) and Honasodu (Shimoga) (13.97° N, 75.57° E). The soil packed in zip lock covers was shade dried for 24 hrs and mixed thoroughly for further studies.

Isolation of actinobacteria from rice rhizhosphere

The soil samples were subjected to serial dilution plate technique (9). Dilutions were prepared from 10^1 to 10^6CFU/mLin autoclaved normal saline and 1 mL of 10^4 and 10^6 CFU/mL dilutions were spread on starch casein agar (SCA) and King's B medium (KB) for isolation of actinobacteria and bacteria, respectively. The pates were incubated at 28 ± 1 °C for 2 days or till the appearance of bacterial colonies. Individual colonies were picked and streaked on respective medium. The isolates were identified as actinomycetes based on colony morphology and color of mycelium (10). A five-letter code was assigned to each rhizobacterial isolate. The first three letters indicated the location (DWR- Dharwad, GVT- Gangavathi, SMG- Shivamogga), while the next two letters denoted the organism type (AM- actinobacteria, Ba- bacteria).

In vitro evaluation of rhizobacterial isolates against *R. solani*

The antagonistic effect of rhizospheric bacteria against highly virulent *R. solani* isolate RS4 (GenBank accession number MK213724) was evaluated using the dual culture technique on Potato carrot Agar (PCA) medium, along with a reference actinobacterial strain AUDT502 (MK367596) and two bacterial strains, *P. fluorescens* IOF 1 (NAIMCC-B-01981) and *B. subtilis* NPPS5 (MT383652) (11). A 5 mm disc of 2-day-old RS4 was placed on both sides of the plate and rhizobacteria were streaked at the centre; control plates had only the pathogen. Plates were incubated at 28 °C and percent mycelial inhibition was recorded after 7 days using formula (Eqn 1.) (12). The experiment was replicated thrice and secondary screening was conducted with best eight isolates.

$$\text{Mycelial inhibition (\%)} = \frac{C-T}{C} \times 100 \quad \text{Eqn. 1}$$

where C- Growth of *R. solani* in absence of rhizobacteria (mm)

T- Growth of *R. solani* in presence of rhizobacteria (mm)

In vivo evaluation of selected rhizobacterial isolates against R. solani under glass house condition

The bioagents found effective during *in vitro* studies were tested against ShB under glasshouse conditions. Seeds of the susceptible rice variety BPT-5204 were sown in earthen pots (15 × 20 cm) filled with autoclaved soil and each pot was fertilized with 2.4 g N, 1.2 g P and 1.2 g K before sowing. The experiment was laid out following a completely randomized block design (CRBD) with 17 treatments and 3 replications per treatment.

Application of potent bioagents

Rhizobacterial suspension (10^8 CFU/mL) was mass multiplied on sterile starch casein broth (SCB) medium and used for seed treatment, foliar spray and a combination of both in glasshouse experiment (13). For seed treatment, seeds were treated with bacterial suspension (5×10^7 CFU/mL) at 10 mL/ g of seed with 0.2 % carboxy methyl cellulose (CMC), air dried and sown (14). For foliar spraying, 2 % culture suspension (10^6 CFU/mL) was sprayed at 15, 30 and 45 days after sowing (DAS); and the combined treatment included both methods.

Pathogen inoculation

The pathogen was mass multiplied on water sedge (*Typha angusta* L.) shoots bits (4.0- 5.0 cm) (2). Rice plants at tillering stage (45 days old) were inoculated with RS4 by placing two typha bits per plant in the middle of the rice tillers. After inoculation, the pots were covered with polythene bags to maintain humidity levels for maximum disease development.

$$\text{RLH (\%)} = \frac{\text{Average lesion length (cm)}}{\text{Plant height (cm)}} \times 100 \quad \text{Eqn. 2}$$

Observations

Observations such as average lesion length, number of dried leaves and plant height were recorded on 60th DAS. Based on lesion length and plant height, relative lesion height percentage was calculated using the formula (Eqn. 2) (15).

Molecular characterization of potent actinobacterial isolates

The chromosomal DNA was extracted using HiPurA® Bacterial genomic DNA purification kit (HiMedia, India) and amplified with 16S rRNA specific primers 27F/1492R (16). PCR was performed in 30 µL reaction mixture containing 1.5 µL each of forward and reverse primer (10 pmol/ µL), 15.0 µL of 1X Emerald GT PCR master mix (Takara, Japan), 3.0 µL template DNA, 1.0 µL of

nuclease free bovine serum albumin (20 mg/mL) and 8.0 µL of molecular grade water. The profile employed for PCR amplification was initial denaturation at 95 ˚C (5 min), followed by 35 cycles of denaturation at 94 ˚C (1 min), annealing at 57.3 ˚C (1 min), extension at 72 ˚C (90 s) and a final extension at 72 ˚C for 10 min. The amplified PCR products were sequenced at NCIM, Pune. The retrieved sequences were edited using BioEdit v 7.2.5 and submitted to NCBI.

Statistical analysis

The data obtained during *in vitro* and *in vivo* experiments were subjected to analysis of variance (ANOVA) for CRBD using M-STAT C programme (17).

Results

Isolation of native bacterial isolates from rice rhizosphere

A total of thirty-two isolates were collected from rhizosphere soil of different rice growing regions of the Karnataka. Among these, 30 showed typical characters of actinobacteria and two were slimy in nature like the fluorescent bacteria. A total of 8 actinobacteria from Gangavathi (GVTAM 1 to GVTAM 8), 13 from Dharwad (DWRAM 1 to DWRAM 13) and 9 actinobacteria (SMGAM 1 to SMGAM 8, SMGAM 10) and 2 bacteria (SMGBa 9, SMGBa 11) from Shimoga were isolated (Supplementary Table 1, Supplementary Fig. 1).

In vitro evaluation of bacterial isolates against *R. solani*

In primary screening, out of 35 isolates comprising of 3 reference strain, 12 actinobacterial isolates and 2 reference strains (AUDT 502 and Pf IOF) showed more than 50 % antagonistic effect against RS4. The per cent mycelial inhibition ranged from 2.22 % to 90.61 %. The highest per cent inhibition was shown by isolates GVTAM 8 (90.61 %), DWRAM 10 (88.38) and reference strain AUDT 502 (87.77 %) (Supplementary Table 2, Supplementary Fig. 2). The top eight isolates viz., GVTAM 8, DWRAM 10, SMGAM 10, SMGAM 1, SMGAM 6, DWRAM 11, SMGAM 3, GVTAM 2 along with reference strains, AUDT 502, Pf IOF and Ba IOF when used for secondary screening yielded similar results confirming the initial findings (Supplementary Table 3, Fig. 1).

Efficacy of potent antagonists against *R. solani* under glasshouse conditions

The two most promising biocontrol agents *viz*., GVTAM 8, DWRAM 10 along with reference strains, AUDT 502 and Pf IOF effective during *in vitro* experiment were further evaluated under glasshouse conditions. The results revealed that, among the three methods of bioagent application, the combined application via seed treatment followed by foliar spray was most effective. Among the rhizobacterial isolates applied, seed treatment + spraying with GVTAM 8 and AUDT 502 was most effective in controlling the disease with least lesion length (1.03

Table 1. Effect of potent bacterial isolates on disease parameters

Tr.No.	Treatment	Lesion length	Relative lesion height (%)	No. of driedleaves per plant
T_1	Seed treatment with GVTAM 8	1.50 (1.58) **	19.90 (4.57) **	2.30 (1.82) **
T_2	Seed treatment with DWRAM10	1.70 (1.64) **	26.27 (5.22) **	2.60 (1.90) **
T_3	Seed treatment with AUDT 502	1.47 (1.57) **	19.05 (4.48) **	2.40 (1.84) **
T_4	Spraying with GVTAM 8	1.53 (1.59) **	20.56 (4.64) **	2.40 (1.84) **
T_5	Spraying with DWRAM 10	1.90 (1.70) **	22.97 (4.90) **	2.83 (1.96) **
T_6	Spraying with AUDT 502	1.67 (1.63) **	20.69 (4.66) **	2.53 (1.88) **
T_7	Seed treatment + spraying withGVTAM 8	1.50 (1.58) **	18.12 (4.37) **	2.13 (1.77) **
T_8	Seed treatment + spraying withDWRAM 10	1.70 (1.64) **	21.81 (4.78) **	2.40 (1.84) **
T_9	Seed treatment + spraying withAUDT 502	1.40 (1.55) **	16.09 (4.13) **	2.07 (1.75) **
T_{10}	Seed treatment + spraying withGVTAM 8 and DWRAM 10	1.47 (1.57) **	18.23 (4.39) **	2.00 (1.73) **
T_{11}	Seed treatment + spraying withGVTAM 8 and AUDT 502	1.03 (1.43) **	14.52 (3.94) **	1.40 (1.55) **
T_{12}	Seed treatment + spraying withDWRAM 10 and AUDT 502	1.33 (1.53) **	15.92 (4.11) **	2.07 (1.75) **
T_{13}	Seed treatment + spraying withGVTAM 8, DWRAM 10 and AUDT 502	1.27 (1.51) **	15.69 (4.09) **	1.92 (1.71) **
T_{14}	Seed treatment + spraying withPf IOF	1.53 (1.59) **	19.53 (4.53) **	1.67 (1.63) **
T_{15}	Hexaconazole @ 0.1 % 1 mL perlitre	1.00 (1.41) **	14.04 (3.88) **	1.58 (1.61) **
T_{16}	Pathogen (Treated control)	3.93 (2.22) **	65.40 (8.15) **	4.00 (2.24) **
T_{17}	Healthy (Untreated control)	0.00 (1.00) **	0.00 (1.00) **	0.00 (1.00) **
	CD value @ 5 %	0.04	0.12	0.07
	S. Em ±	0.01	0.04	0.26
	CV value	1.81	1.26	2.57

** Square root transformed values

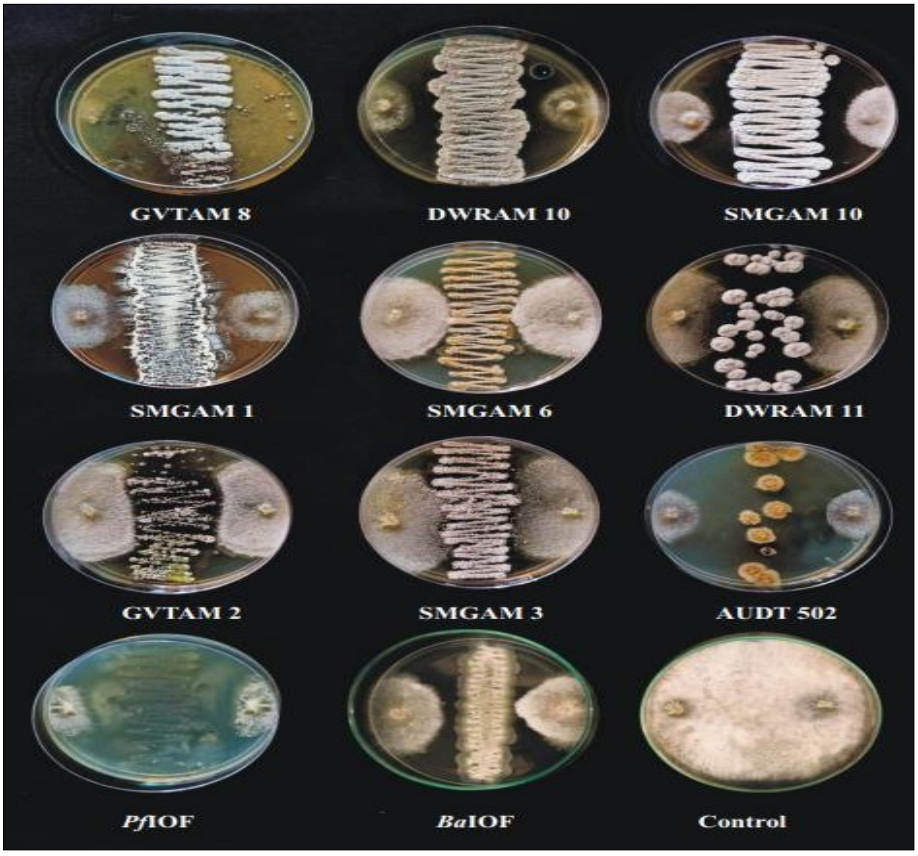

Fig. 1. *In vitro* evaluation (secondary screening) of actinobacteria against *R. solani.*

cm), relative lesion height (14.52 %) and number of dried leaves (1.40) and was statistically on par with hexaconazole treatment with an average lesion length of 1.00 cm. The next best was combined application of GVTAM 8, DWRAM 10 and AUDT 502, recording an average lesion length of 1.27 cm (Table 1).

Molecular characterization of potent antagonists

The DNA from two best isolates *viz.*, GVTAM 8 and DWRAM 10 when subjected to PCR analysis with 16S rRNA specific primer yielded an amplicon of approximately 1.5 kb size. The GVTAM 8 and DWRAM 10 sequences were submitted to NCBI with accession number OQ512000 and OQ512154, respectively. The isolate, GVTAM 8 showed maximum nucleotide similarity of 98.06 % with *Streptomyces cinnabarinus* (GenBank accession number NR_115667) and DWRAM 10 showed the maximum similarity of 98.21 % with *Streptomyces pseudogriseolus* (GenBank accession number NR_043835) based on BLAST search, whereas AUDT 502 had been previously identified as *Streptomyces rimosus* (GenBank accession number MK367596).

Discussion

Sheath blight is one of the most devastating diseases affecting rice worldwide. Biological control such as use of native microbiome is particularly promising, but its success depends on identifying antagonist strains that can suppress the pathogen across diverse conditions, establish well in the rhizosphere and simultaneously enhance crop growth and yield (18).The plant microbiome comprises the entire microbial community associated with plants, within which PGPR form a major group residing in the rhizosphere (19).

These microbes colonize different plant compartments, forming complex networks that contribute to plant growth directly by enhancing nutrient availability and indirectly by inducing systemic resistance against phytopathogens, while also improving nutrient uptake, disease resistance and environmental adaptability (20, 21). The use of actinobacteria in crop protection has recently increased due to their ubiquitous presence in plant environments and their multifaceted roles as PGPR, including competition with phytopathogens, interference with toxin production and enhancement of plant growth through mechanisms such as phytohormone production, phosphate solubilization, siderophore production, rhizosphere engineering, quorum sensing, inhibition of biofilm formation, volatile organic compound production and promotion of beneficial plant-microbe symbioses (22-24).

In this study, several bacterial antagonists were recovered from rhizosphere soil of rice. For the selective isolation of actinobacteria, SCA was used, as this medium supports actinobacterial growth due to its high carbon-to-nitrogen (C: N) ratio and the presence of complex macromolecules such as starch and casein (25). King's B medium was used for fluorescent bacteria since presence of trace elements in this media supports the growth of such bacteria (26).

Identification of actinomycetes based on colony morphology and pigment production has been a longstanding practice. In a related study, *Streptomyces* spp. effective against ShB was phenotypically identified based on characters such as spore morphology, aerial and substrate mycelia, pigment production and colony characteristics (27). In our study, colony morphology and pigmentation were sufficient to distinguish among isolates. The slimy texture of fluorescent *Pseudomonas* clearly differentiated them from actinobacterial isolates, which exhibited colony colours including white, grey, blue, pink, brown and yellowish hues.

The antagonistic effect of different bioagents was assessed against *R. solani* by dual culture technique and glasshouse assays. Among the treatments, the combination of seed treatment and foliar application of actinobacteria proved most effective under glasshouse conditions. In a similar study, 225 native actinobacteria were isolated from rice rhizosphere through serial dilutions from different soil samples. Of these, six isolates, when applied in combination, exhibited more than 85 % inhibition of *R. solani* (11).

The suppressive effects of actinobacteria on pathogen growth have previously been attributed to multiple mechanisms, including antibiosis, nutrient competition and production of cell wall-degrading enzymes, nitrous oxide, siderophores, volatile compounds and quorum quenching (28-30). In the present study, hydrogen cyanide (HCN) and siderophore production were identified as the key inhibitory mechanisms against *R. solani* (data not shown). HCN disrupts the pathogen's electron transport chain, thereby halting ATP synthesis and leading to cell death (31). Meanwhile, siderophores, the iron-chelating compounds enhance the solubility and availability of ferric ions (Fe^{3+}) to the host plant. This deprives the pathogen of essential iron required for its survival (32).

The top two rhizobacterial isolates in this study were closely related to *Streptomyces* species. Several *Streptomyces* strains, including *S. philanthi*, *S. aurantiogriseus*, *Streptomyces* strain PM5 and *S. padanus*, have previously demonstrated strong antagonistic activity against *R. solani* (7, 28, 33-35). *Streptomyces* spp. is well known for their applications as biofertilizers in agricultural soils, biocontrol agents for plant disease management and producers of valuable bioproducts in the medical field, biofertilization, bioremediation and biofuel (36). Their ability to produce antibiotics and a wide range of extracellular enzymes particularly those that degrade fungal cell walls makes them highly effective biocontrol agents. These enzymes facilitate the lysis of fungal pathogens either individually or in synergy with other biocontrol agents (37, 38). Recent studies have emphasized the significant potential of *Streptomyces* spp. in integrated disease management (IDM) programs (35). The consistent effectiveness of these isolates against *R. solani* indicates their potential to reduce dependence on chemical pesticides and be integrated into IDM strategies for sustainable ShB management. Future research should emphasize formulation, large-scale commercialization and characterization of biosynthetic gene clusters to develop novel biopesticides (39).

Conclusion

This study identified native *Streptomyces* spp. from rice rhizosphere soils in Karnataka with strong antagonistic activity against *Rhizoctonia solani*. Among 32 isolates, GVTAM 8 (*S. cinnabarinus*) and DWRAM 10 (*S. pseudogriseolus*) showed superior efficacy under *in vitro* and glasshouse conditions, particularly with combined seed treatment and foliar application. These findings highlight their potential as biocontrol agents for sustainable rice cultivation, warranting further field validation and consortium development for effective ShB disease management and yield improvement.

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Authors' contributions

TBS, AM, PSK and HSV carried out conceptualization and methodology. Formal analysis and investigation done by TBS and AM. AM and TBS carried out original draft preparation. TBS, AM, PSK, RA carried out review and editing. TBS, AM, PSK, HSV and JPN carried out supervision. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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