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New rhizobacteria strains with effective antimycotic compounds against rhizome rot pathogens and identification of genes encoding antimicrobial peptides

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A B S T R A C T

Bacterial strains were isolated from turmeric rhizosphere and 18 of them exhibited plant growth promoting traits and ability to solubilize mineral. They also showed the ability to concurrently suppress the growth of multiple fungal phytopathogens under *in vitro* assay. An isolate of *Bacillus safensis*, produced indole 3-acetic acid, NH_3, HCN, siderophore, and cell wall degrading enzymes (cellulase, protease and pectinase) and exhibited remarkable suppression of various fungal pathogens infecting turmeric *viz.*, *Pythium myriotylum, P. aphanidermatum*, *Colletotrichum gloeosporioides, C. capsici, Macrophomina phaseolina* and *Fusarium oxysporum* under *in vitro* conditions. In a subsequent greenhouse experiment, turmeric was inoculated with these fungal pathogens and in treatments with *B. safensis* the Disease Index (DI) was significantly reduced compared to the fungicide treatment. The greenhouse trials evidently demonstrated that the rhizome rot incidence in turmeric treated with *B. safensis* decreased by 84.61%, compared to fungicide treatments. We searched for the presence of biosynthetic genes that encode for the production of antimicrobial peptides (AMP) in the shortlisted rhizobacterial strains and the *B. safensis* strain, with the most biocontrol efficiency showed the presence of the genes encoding bacillomycin, surfactin and iturin. The dominance of putatively encoded biosynthetic genes reaffirmed the robust biocontrol potential of this strain for protection against a broad array of fungal phytopathogens. Our findings indicated that the growth promotional and antifungal potential of *B. safensis* strain, IISR-TB4 (NCBI- MT192800) could be further exploited to reduce the dependence on fungicides for sustainable turmeric cultivation.

Authors' contributions

R Praveena: Conceptualization, Visualization, Methodology, Writing-Editing, K Srekha: Investigation, R Revathy: Investigation, K P Subila: Investigation, Priya George: Investigation, V Srinivasan: Software, Validation, Formal analysis, C Sarathambal: Data curation, R Dinesh: Supervision, Methodology, Writing – original draft

1. Introduction

Plant growth promoting rhizobacteria [PGPR] consist of a group of root colonizing bacteria that promote plant growth through direct and indirect mechanisms and are established alternatives to synthetic agrochemicals and other conventional agricultural practices (Gouda et al., 2018; Grobelak et al., 2018; Nagargade et al., 2018). Besides growth promotion, disease suppression by PGPR is an important facet of research owing to the indiscriminate use of chemicals that have deleteriously affected crop quality, soil quality and the environment (Meena et al., 2019). Over use has made several phytopathogens tolerant to broad-spectrum fungicides, urging the need for heavier doses and new generation fungicides with different modes of action (Raymaekers et al., 2020). Ergo, the use of PGPR for biological control is considered a benign option for negating such issues in crop production.

Majority of research work with PGPR as antagonists have focused on assessing the efficacy of a PGPR strain against a single disease (Li et al., 2011; Kalantari et al., 2018). But under field conditions the crops are susceptible to several pathogens and hence, there is a compulsive need to identify PGPR strains effective against multiple diseases. However, only a limited broad spectrum PGPR strains are commercially available *viz.*, Kodiak (*Bacillus subtilis*) for the management of *Rhizoctonia* spp.,

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Fusarium spp. and *Aspergillus* spp., Integral (*B. subtilis*) for the management of *Rhizoctonia* spp. and *Fusarium* spp., and Serenade (*B. subtilis*) for *Botrytis* spp., *Sclerotinia* spp., *Xanthomonas* spp. and *Erwinia* spp. (Liu et al., 2017; Naseri and Younesi 2021).

Antibiosis is considered as the major mechanism employed by PGPR through the production of phenazines, 2,4-diacetylphloroglucinol (2,4-DAPG), pyoluteorin, pyrrolnitrin, zwittermycin A, kanosamine, fengycin, iturin, surfactin etc. Earlier reports include production of zwittermycin and kanosamine by *B. cereus* strain for the biological control of *Phytophthora medicaginis* (Stabb et al., 1994), fengycin by *B. subtilis* for *Rhizoctonia* diseases (Deleu et al., 2008) and iturin by *B. amyloliquefaciens* for *R. solani* (Yu et al., 2002). While the antagonistic effects of PGPR to phytopathogens has been reported in numerous crops, information on PGPR for their simultaneous suppression of multiple diseases caused by different phytopathogens in turmeric is sparse. In the present study, we isolated an array of PGPR from turmeric cultivated in different geographical locations of India and the most promising isolates were studied for their AMP production and subsequently studied for their biocontrol potential against multiple pathogens infecting turmeric.

Turmeric (*Curcuma longa* L.) known as the golden spice is one of the most widely cultivated spice crops in many tropical and sub-tropical regions of the world. Diseases are considered as one of the major production constraints in all turmeric-growing tracts and rhizome rot and foliar diseases are considered to be a serious yield limiting factors. Turmeric is infected by several pathogens of which, *Pythium aphanidermatum, P. myriotylum*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Macrophomina phaseolina* causing rhizome rot, *Colletotrichum capsici* and *C. gloeosporioides* causing leaf disease are common. In fact, yield losses due to phytopathogens, especially rhizome rot has been estimated to be as high as 60% in nursery and field conditions and losses due to the disease could go as high as 50–80% in storage (Rajalakshmi et al., 2016). Crop loss assessment studies indicated that leaf blotch diseases resulted in 21.6–32.5% loss of biomass and 24.5–32.0% loss of fresh rhizomes (Panja et al., 2001), whereas 34.0–57.0% yield loss was reported due to leaf blight disease caused by *Colletotrichum* spp.

Deploying tolerant varieties and use of conventional fungicides have been the common strategies followed for disease management in turmeric. Harmful effect of agrochemicals on environment and non-target organisms has been well documented (Naseri and Hemmati, 2017). However, with the current focus on fewer chemical inputs, PGPR are considered as green alternatives for sustainable crop cultivation. Hence, the overall objective of the study was to identify PGPR strains with antagonistic potential against a broad spectrum of phytopathogenic fungi infecting turmeric and identify multiple traits related to plant growth promotion. A major objective was also to predict the genes encoding AMPs, which could at least partially explain the biocontrol activities of the promising strains.

2. Materials and methods

2.1. Soil sampling

Rhizosphere soils were collected from major Zingiberaceous crop (turmeric and ginger) growing locations of India (Kozhikode and Wayanad, Kerala state; Mysore and Chamarajanagar, Karnataka state; Coimbatore and Erode, Tamil Nadu state, Guntur and Krishna, Andhra Pradesh state & Nizamabad and Jagtial, Telangana state). Soil strongly adhering to the roots and within the space explored by the roots samples were collected from randomly selected turmeric and ginger plants. The samples were immediately transferred to ice box and under laboratory conditions the live plant material and roots were removed prior to biochemical/microbial parameter analysis of the samples. A portion of each sample was stored at 4 °C for not more than one week before analyses. The location details are given in Table S1.

2.2. Isolation and screening of bacterial isolates

Isolation of bacteria was done using the serial dilution technique (up to 10^{-10}). The suspension from different dilutions was pour-plated in different media *viz*., nutrient agar (NA), Jensen's, Burk's (for detection of nitrogen fixing organisms) and Pikovskaya's (for detection of phosphate solubilizing microorganisms) and incubated at 28 ± 2.0 °*C* for 2–4 days. Sub-culturing of individual colonies was done in NA medium. All the isolates so obtained, were tested for *in vitro* antagonism against *Pythium aphanidermatum, P. myriotylum*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Macrophomina phaseolina*, *Colletotrichum capsici* and *C. gloeosporioides* isolated from turmeric using dual-plate culture method (Berg et al., 2005). This involved placing a mycelial plug of actively growing pathogen on PDA plates and streaking the individual bacterial isolates, 2.0 cm away on either side of the plug followed by incubation at 28 ± 2.0 °*C* until the fungal growth in the control fully covered the plate surface. *In vitro* assay was done in triplicates and repeated to confirm the results.

The mycelial growth inhibition percent (I) was calculated as,

$$I = [C - T / C] \times 100$$

where, T and C are the radial growth of the pathogen in treatment and control, respectively. From this experiment, 18 bacterial isolates that showed more than 30.0% suppression of at least one test pathogen were used for further studies. Shortlisted 18 bacterial isolates were also studied for an array of phenotypic characters using the methods of Holt et al. (1994) and Tindal et al. (2007) and plant growth promoting (PGP) traits.

2.3. In vitro screening for plant growth promoting (PGP) traits of shortlisted bacterial isolates

The shortlisted bacteria were evaluated for the production of different PGP traits *viz*., IAA, NH_3, HCN, siderophore and hydrolytic enzymes. All the assays were carried out with three replications and the experiments were repeated twice.

2.3.1. Indole-3 acetic acid (IAA) production

The 18 isolates were evaluated for IAA following the procedure of Sawar and Kremer (1995). Briefly, 5.0 mL of sterile peptone/tryptone yeast extract broth was inoculated with 50.0 µL of cell suspension and incubated in the dark 72 h at 28.0 ± 2.0 °*C*. Then, 1.5 mL of the suspension was centrifuged and to 1.0 mL of the supernatant equal volume of Salkowski reagent was added and incubated in the dark (37 °*C* for 1.0 h). Appearance of red color indicated that the bacterial strain produced IAA. The IAA was quantified by reading the color intensity at 530 nm in a UV–vis spectrophotometer (Shimadzu) and the amount of IAA released was calculated from a standard graph prepared using known quantities of pure IAA.

2.3.2. Ammonia (NH_3) production

The method described by Cappuccino and Sherman (1992) was used to determine NH_3 production. Briefly, 50.0 µL of bacterial cell suspension was transferred to 30.0 mL of peptone broth (4.0%), incubated at 25.0 °*C* for 72.0 h and after incubation, 1.0 mL of Nessler's reagent (Hi Media) was added. The development of yellow to brown precipitate indicated the release of NH_3.

2.3.3. HCN production

HCN production was estimated by the method of Kloepper et al. (1991) with modifications (Dinesh et al., 2015). In short, 25.0 µL of log phase bacterial culture was inoculated into 5.0 mL King's B broth supplemented with 4.40 g L^{-1} of glycine taken in 30.0 mL sterile glass vials. In the vials, filter paper strips soaked in picric acid solution were inserted and sealed using parafilm, placed on a shaker and incubated for

72.0 h. Change in color of the filter paper strips to red suggested that the bacterial isolate was positive for HCN production.

2.3.4. Siderophore production

Siderophore production was measured qualitatively by spot inoculating the test bacterium on Chrome Azurol S agar medium and incubation at 28.0 ± 2.0 °*C* for 3–5 days. Appearance of yellowish orange halo enclosing the spot was considered as positive reaction (Schwyn and Neilands, 1987).

2.3.5. Production of hydrolytic enzymes

For determining α-amylase production, one loopful of the cell suspension was streaked on starch agar plate incubated at 28 ± 2 °*C* for 2 days and starch hydrolysis was detected by drenching the iodine solution on the inoculated plates. The isolates that developed a clear hydrolysis zone in the agar plate were recorded. For estimating protease, one loop full of the bacterial suspension was streaked on casein agar plate and incubated at 28 ± 2 °*C* for 2 days and the plates were examined for clear zone around the streak. Pectinase production was determined by streaking one loopful of the cell suspension on the medium (1.0% pectinase in basal medium consisting of $NaNO_3$-1.0g, K2HPO4 -1.0g, KCl-1.0g, $MgSO_4$-0.50 g, yeast extract-0.50 g, glucose-1.0 g, agar-15.0 g, distilled water-1.0L) and incubating for 5 days at 28 ± 2 °*C*. Gram's iodine solution was poured into the pectin agar and zone of clearance was observed. Cellulase production was estimated by streaking one loopful of suspension on the basal medium (same as above) containing cellulose (1.0%) and incubating for 5 days at 28 ± 2 °*C*. Thereafter, Congo red solution (0.01%) was added for 15 min followed by destaining using NaCl (1.0%) for 5 min. Appearance of clear zone indicated cellulase enzyme production. All the assays were done in triplicates using the procedure of Cappuccino and Sherman (1992) and repeated to confirm the results.

2.4. Mineral solubilization assay

2.4.1. Phosphorus (P), potassium (K), zinc (Zn) and silica (Si)

P solubilization efficiency was estimated using Pikovskaya agar medium (Zaidi et al., 2006). One loop full of the 24-h bacterial cultures were spot inoculated on the Pikovskaya culture plate separately. The plates were incubated at 26±2 °C for 96 h and the formation zone of clearance around the bacterial colony indicated the solubilization of mineral phosphorous. For determining K solubilization, Aleksandrov medium containing mica (Hu et al., 2006) was used. Inoculated plates were incubated at 26±2 °C for 3 days and the solubilization efficiency was calculated by measuring the clear zones.

Zn solubilization capability was determined using mineral salt agar amended with 0.5% insoluble Zinc oxide (ZnO) (Venkatakrishnan et al., 2003). The actively growing culture (5 μL) was spot inoculated onto the medium, the plates were incubated at 26±2 °C and zone of solubilization was measured 15 days after inoculation (DAI). Silica solubilization was determined using the protocol of Waqas et al., (2014). Each bacterial isolate was spot inoculated on silicate medium and solubilization around the colony was measured after seven DAI. All the mineral assays were done in triplicates and repeated to confirm the results. The P, K, Zn and Si solubilization efficiency (SE) was determined and expressed as a ratio between the diameter of the halo zone and the diameter of the bacterial colony.

2.5. Identification of shortlisted isolates using 16S rRNA

Briefly, the genomic DNA from the short listed bacterial strains was isolated (Sambrook and Russell, 2001) and the16S rRNA gene was amplified using the 27F-1492R primer pair (Ni et al., 2015; Turner et al., 1999; Weisburg et al., 1991) i.e. 27F (5′-AGAGTTTGATCCTG GCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) in 25.0 μL of reaction mixture containing 1x buffer with $MgCl_2$ (2.5 μL), dNTPs mix (2.0 μL), Taq polymerase (0.3 μL),0.5 μM of each primer, and template DNA (2.0 μL) and nuclease free water (17.2 μL). The PCR conditions involved initial denaturation for 2 min at 94 °*C* followed by 3 0 cycles of denaturation (1.0 min at 94°*C*), annealing (1.0 min at 52 °C, extension (1.0 min at 72 °*C*) followed by final extension (10 min at 72 °*C*). The amplified products were analyzed on agarose gel (1.5%) in presence of ethidium bromide along with a 1.0 kb ladder as size standard. The bands were visualized under UV and photographed using Gene Sys software. The PCR products were sequenced at M/s Agrigenome, Kochi, Kerala, India.

Following BLAST, the sequences were compared with registered sequences in the GenBank database using NCBI Blast server (http://www.ncbi.nlm.nih.gov). The analysis indicated 99–100% similarity with 16S rRNA gene sequences of bacterial strains already available in NCBI. The 16S rRNA sequences of these bacterial strains were deposited in the GenBank database of NCBI. Phylogenetic tree and evolutionary distances (Knuc) were developed using the neighbour-joining method by employing boot strapping (1000 replicates). We used the Kimura 2-parameter model, wherein, the sequence of the most promising bacterial strains and other PGPR reported in the literature were aligned using MUSCLE fused in MEGA 7.0 (Kumar et al., 2016).

2.6. Molecular detection of antimicrobial peptides (AMP) coding genes of shortlisted PGPR

The total genomic DNA was isolated from the shortlisted eight rhizobacteria and were used for the detection of biosynthetic genes that encode for the production of antibiotics viz., bacillomycin D, fengycin, iturin, surfactin, 2,4-diacetylphloroglucinol (DAPG), pyoluteorin (PLT), pyrrolnitrin (PRN), the volatile metabolite, hydrogen cyanide (HCN) and the production of cellulase enzyme. Gene specific oligonucleotide primers were synthesized and the primer sets and PCR amplification conditions are given in Table S2. PCR amplifications were performed in25.0 μL of reaction mixture containing 1x buffer with $MgCl_2$ (2.5 μL), dNTPs mix (2.0 μL), Taq polymerase (0.3 μL),0.5 μM of each primer, and template DNA (2.0 μL) and nuclease free water (17.2 μL). PCR reactions were performed using an automated Thermal Cycler Biorad (T100). The amplified products were analyzed on agarose gel (1.5%) in presence of ethidium bromide along with a 1.0 kb ladder as size standard. The bands were visualized under UV and photographed using Gene Sys software. Amplicons were gel purified using commercial column-based purification kit (Invitrogen, USA) and sequencing was performed with forward and reverse primers in ABI 3730 XL cycle sequencer. Forward and reverse sequences were assembled and contig was generated after trimming the low quality bases and sequence analysis was performed using NCBI database and based on maximum identity score top most sequences were utilized for multiple sequence alignment (Clustal W).

2.7. Green house evaluation of promising PGPR strains for disease suppression in turmeric

Earthen pots (capacity 10 kg) were filled with sieved soil (<2 mm). The soil was an Inceptisol with organic C content of 14.0 g kg^{-1}; mineral N – 40 mg kg^{-1}; Bray P – 5.5 mg kg^{-1}; Exchangeable K– 90 mg kg^{-1} and pH of 5.12. While planting, seed rhizomes (~25–30 g) of turmeric (variety: IISR- Prathibha with minimum two sprouts) were placed in shallow pits at 3.0–3.5 cm depth and concealed with soil. The treatments adopted in the study are described in Table S3. For application of PGPR, the rhizomes were immersed for 1.0 h in the bacterial suspension (1×10^7 CFU mL^{-1}) containing 1.0% starch solution and shade dried for 24 h prior to planting. Booster doses of each PGPR was given at 30, 60 and 90 days after planting (DAP) by spraying and drenching @ 1.0 L pot^{-1} (10^7 CFU mL^{-1}). For preparation of bacterial cultures, the freshly grown culture was inoculated in 250 mL nutrient broth and incubated in a rotary shaker at 26 ± 2 °*C* for 48 h. Thereafter, it was diluted with sterile distilled water to get a concentration of 10^7 CFU mL^{-1}. All the pots were

applied with recommended dose (RD) of NPK for turmeric (60–50–120 kg ha^{-1}). N was applied as urea, P as rock phosphate (RP) and K as muriate of potash (MOP). The plants were then simultaneously challenged with the major rot pathogens of turmeric *Pythium aphanidermatum* and *P. myriotylum*.

Two independent experiments were conducted in completely randomized design, the first experiment for testing the biocontrol potential of selected PGPR against soft rot pathogen and the treatments included the promising PGPR *B. safensis* and *B. cereus*, a fungicide-metalaxyl–mancozeb –applied as both seed treatment and soil drench, untreated control and absolute control (Table S3). The second experiment for evaluating the selected PGPR against foliar pathogen and the treatments included the promising PGPR *B. safensis* and *B. cereus*, a fungicide-carbendazim -mancozeb –applied as both seed treatment and foliar spray, untreated control and absolute control (Table S3). The treatments were replicated six times.

Briefly, inoculum of *Pythium* spp was prepared in sand-maize medium, (100 g of thoroughly washed sand, 5.6 g of ground corn grains and 40 mL of H_2O were taken in 250 mL conical flask, mixed well and sterilized at 121 °*C* for 1 h. The sterilization was repeated after 24h for 1.0 h) and 5 mm discs of *Pythium* spp were cut from growing edges of 72h grown culture and transferred to sterile sand-maize medium. The flasks were incubated at 24 ±1 °*C* for 7 days and shaken every day to ensure uniform distribution of the fungus. The pathogen was inoculated @ 100 g/10 kg soil at 30 DAP. During inoculation the top layer of soil in the pots was gently raked and the pathogen inoculum was mixed well with soil. Second set of plants were inoculated with the foliar pathogen *Colletotrichum capsici* and *C. gloeosporioides*. Prior to inoculation of these foliar pathogens, the matured leaves of turmeric (during 4–5 leaves stage/45 DAP) were surface sterilized with 70% alcohol and after 2h the sterilized leaves were sprayed with the mixed conidial suspension (1×10^6 conidia mL^{-1}) of the fungus using an atomizer. The suspension also contained an emulsifier (Tween 20, 0.05%). The relative humidity in the green house was 80–90%, while the temperature hovered between 24 and 33 °*C*.

Observations on sprouting was recorded at 20 DAP. Six replicates were maintained for each treatment and observations on number of tillers, yellowing of tillers, soft rot and foliar disease incidence and rhizome yield pot^{-1} (240 DAP) were recorded. The severity of foliage symptoms (yellowing & leaf diseases) was assessed on a 0–5 scale, wherein, 0 = no disease; 1 = 1–25% yellow leaves or leaf spot/blight symptoms; 2 = 26–50% yellow leaves or leaf spot/blight symptoms; 3 = 51–70% yellow leaves or leaf spot/blight symptoms; 4 = 71–90% yellow leaves or leaf spot/blight symptoms; and 5 = 100% yellow leaves or leaf spot/blight symptoms (complete blighting of leaves). Soft/rhizome rot (*P. myriotylum*) was assessed on a 0–4 scale, wherein 0 = no infection; 1 = 1–25% infected area; 2 = 26–50% infected and rotten area; 3 = 51–75% rotten area; 4 = 76–100% rotten area (mortality). The plants were scored at weekly intervals after challenge inoculation. This greenhouse experiment was repeated twice to confirm the results.

The percent disease index (PDI) was calculated as follows:

$$PDI = \left(\sum \text{ofalldiseaseratings} / \text{totalnumberofplantratings} \times \text{highestnumericalrating} \right) \times 100$$

2.8. Statistics

The significance among treatments was estimated by one-way ANOVA. To improve the homogeneity, all PDI values were arcsine square root transformed before further analysis. In case the F values were significant, the means were compared using the Least Significance Test (LSD) at $P < 0.05$.

3. Results

3.1. Isolation and initial screening of bacterial isolates

270 bacterial isolates were isolated, from which we selected 18 isolates based on their capacity to inhibit >30.0% of at least one of the major turmeric pathogens *in vitro* (Table 1, Fig. S1). The shortlisted isolates belonged to the genera *Bacillus* spp. (10), *Pseudomonas* spp. (3), *Ochrobactrum* sp., *Phytobacter* sp., *Stenotrophomonas* sp., *Paenibacillus* sp., and *Micrococcus* sp. (1 each) (Table S4, Fig. S2). Among the 18 isolates, 8 isolates *viz*., IISR TB1, IISR TB4, IISR TB5, IISR GB1, IISR GB2, IISR GB3, IISR GB5(1) and IISR GB7(3) showed more than 30% inhibition against the four rhizome rot pathogens tested. Among these, IISR TB4 and IISR GB7(3) showed maximum inhibition (90%) of *P. aphanidermatum, P. myriotylum, M. phaseolina* and *F. oxysporum*. In case of foliar pathogens, 12 bacterial isolates showed more than 30% inhibition of *C. gloeosporioides* and *C. capsici*. Among these, IISR TB4 and IISR GB7(3) showed maximum inhibition of 89.02 and 87.58%, respectively.

3.2. In vitro screening for plant growth promoting (PGP) traits of shortlisted bacteria

Except for three isolates [IISR GB2, IISR GB6(1) and IISR GB7(2)], all the tested isolates were able to produce IAA (Table S5), with maximum of 8.14 ± 0.02 and 7.12 ± 0.01 μgmL^{-1} for *B. safensis* and *B. cereus* respectively (Table 2). Out of the 18 isolates, 10 isolates including *Bacillus* and *Pseudomonas* showed HCN production, whereas only 7 isolates produced siderophores. With regard to hydrolytic enzymes, 9 isolates were positive for pectinase and protease, 10 for catalase and none of the isolates produced α-amylase (Table S5). Extracellular protease activity of *B. safensis* and *B. cereus,* was 1.7 ± 0.01 and 1.2 ± 0.01 AU, respectively (Table 2). The higher siderophore producing capacity of *B. safensis* was 1.5 ± 0.03 AU (Table 2). *In vitro* plant growth promoting traits of *B. safensis* are shown in Fig. S3.

Out of the 18 shortlisted isolates, nine could solubilize phosphorus, seven solubilized zinc, and five potassium and the isolates IISR GB7 (3) – *Bacillus cereus* and IISR TB4- *Bacillus safensis* showed the maximum solubilization efficiency (Table 2 and Table S6). The two most promising isolates IISR TB4 and IISR GB7 (3) were identified as *B. safensis* (NCBI-MT192800) and B. cereus (NCBI- MT192803) respectively. The phylogenetic tree constructed using 16S rRNA sequences indicated the relationship between our strain, IISR-TB4 MT192800 (B. saf*ensis*) and other PGPR (Fig. S5).

3.3. Molecular detection of antibiotic coding genes produced by shortlisted PGPR

The bacillomycin D gene cluster primers amplified the specific 875 bp product (Table 3) in *B. safensis* IISRTB4 and *B. cereus* IISR GB7 (3). BLASTp analysis of the putative precursor peptide gene showed highest homology to the bacillomycin D operon of *B. subtilis* (AY137375). The fengycin biosynthetic gene cluster primers FEND1F/FEND1R amplified the expected 964bp product in three PGPR strains. Iturin gene (647 bp) was detected in *B. pumilus* IISR TB1, *B. cereus* IISR TB2 and *B. safensis* IISR TB4. The Phl2a/Phl2b primers specific for DAPG amplified the expected 745 bp product in *P. aeruginosa* IISR TB7, while pyrrolnitrin and pyoluterin gene-specific primers amplified the expected 719 bp and

Table 1
Shortlisted rhizobacteria based on *in vitro* antagonism on rhizome rot and foliar fungal pathogens infecting turmeric.

Shortlisted PGPR isolates	Percent inhibition (%)					
	P.aphanidermatum	P. myriotylum	C. capsici	C. gloeosporioides	M.phaseolina	F. oxysporum
TB1-*Bacillus pumilus*	40.07 ± 0.55 ^{de}	37.29 ± 0.52 ^{d}	36.53 ± 0.69 ^{cd}	29.20 ± 0.58^{ef}	59.91 ± 0.43 ^{cd}	57.91 ± 0.53 ^{c}
TB2- *B. cereus*	41.52 ± 0.80 ^{d}	43.52 ± 0.45 ^{c}	36.23 ± 0.50 ^{cd}	31.57 ± 0.51^{e}	29.67 ± 0.53^{gh}	31.00 ± 0.54 ^{f}
TB3- *B. pumilus*	33.15 ± 0.37^{fg}	32.48 ± 0.50 ^{d}	24.00 ± 0.58 ^{f}	21.00 ± 0.47 ^{g}	0^{i}	0^{g}
TB4- *B. safensis*	92.35 ± 0.48^{a}	90.01 ± 0.63 ^{a}	89.02 ± 0.47 ^{a}	87.69 ± 059 ^{a}	88.32 ± 0.68 ^{a}	87.99 ± 0.69 ^{a}
TB5- *Pseudomonas* spp	62.26 ± 0.81 ^{b}	62.93 ± 0.69 ^{b}	32.60 ± 0.52 ^{de}	31.27 ± 0.66^{e}	52.85 ± 0.52^{e}	49.00 ± 0.71 ^{d}
TB6- *Ochrobactrum intermedium*	0^{j}	21.00 ± 0.47^{ef}	40.65 ± 0.74 ^{c}	40.32 ± 0.50 ^{c}	0^{i}	44.52 ± 0.59 ^{de}
TB7-*P.aeruginosa*	30.74 ± 0.43^{fg}	34.41 ± 0.52 ^{d}	32.73 ± 0.49 ^{de}	37.73 ± 0.57 ^{cd}	24.00 ± 0.63^{h}	21.33 ± 0.48^{i}
GB1-*P.aeruginosa*	65.96 ± 0.54 ^{b}	65.63 ± 0.68 ^{b}	0^{g}	0^{h}	54.90 ± 0.52 ^{de}	68.62 ± 0.62 ^{b}
GB2- *B. cereus*	65.31 ± 0.58 ^{b}	61.31 ± 0.67 ^{b}	61.55 ± 0.73 ^{b}	61.00 ± 0.47 ^{b}	60.33 ± 0.41 ^{c}	67.33 ± 0.62 ^{b}
GB3-*Phytobacter diazotrophicus*	52.12 ± 0.53 ^{c}	47.79 ± 0.50 ^{c}	33.74 ± 0.56 ^{de}	29.07 ± 0.64^{ef}	42.56 ± 0.63 ^{f}	41.33 ± 0.53^{e}
GB4- *B. subtilis*	20.67 ± 0.41^{i}	26.00 ± 0.63^{e}	0^{g}	0^{h}	42.60 ± 0.47 ^{f}	39.94 ± 0.69^{e}
GB5(1)-*Stenotrophomonas maltophilia*	31.82 ± 0.51^{fg}	36.82 ± 0.50 ^{d}	20.66 ± 0.41 ^{f}	24.00 ± 0.63^{fg}	0^{i}	0^{g}
GB5(2)-*Paenibacillus* spp	28.37 ± 0.53^{gh}	35.04 ± 0.66 ^{d}	31.33 ± 0.67 ^{de}	24.33 ± 0.58^{fg}	0^{i}	0^{g}
GB6(1)- *Bacillus* spp	31.33 ± 0.67^{fg}	0^{g}	30.14 ± 0.65^{e}	0^{h}	0^{i}	32.61 ± 0.47^{f}
GB6(2)- *B. marisflavi*	31.00 ± 0.47^{fg}	0^{g}	30.74 ± 0.43^{e}	32.07 ± 0.33 ^{de}	30.53 ± 0.58^{gh}	29.53 ± 0.56 ^{f}
GB7(1)- *Micrococcus luteus*	34.15 ± 0.58 ^{f}	36.15 ± 0.53 ^{d}	0^{f}	31.33 ± 0.67^{e}	0^{i}	0^{g}
GB7(2)-*Cytobacillus firmus*	35.06 ± 0.58^{ef}	34.40 ± 0.37 ^{d}	24.00 ± 0.63 ^{f}	21.00 ± 0.47 ^{g}	0^{i}	0^{g}
GB7(3)- *B. cereus*	90.09 ± 0.34 ^{a}	89.75 ± 0.61 ^{a}	87.58 ± 0.52 ^{a}	87.58 ± 0.59 ^{a}	82.50 ± 0.41 ^{b}	68.50 ± 1.03 ^{b}

In a column, means followed by the same letter are not significantly different at $P < 0.05$.

Table 2
Plant growth promoting (PGP) traits of *Bacillus safensis* (IISR TB4) & *B. cereus* [IISR GB7(3)] under *in vitro*.

PGP traits	Response units[a]	
	Bacillus safensis	Bacillus cereus
Indole acetic acid production (μg/ml)	8.14 ± 0.02	7.12 ± 0.01
Tricalcium phosphate solubilization (SE)	5.87 ± 0.02	6.67 ± 0.03
Zinc oxide solubilization (SE)	7.83 ± 0.03	6.94 ± 0.05
Mica solubilization (SE)	5.71 ± 0.03	3.92 ± 0.02
Magnesium trisilicate solubilization (SE)	–	5.86 ± 0.01
Ammonia production	+	–
HCN production	++	++
Siderophore production (AU)	1.5 ± 0.03	–
Amylase activity (AU)	–	–
Protease activity (AU)	1.7 ± 0.01	1.2 ± 0.01
Pectinase activity (AU)	1.8 ± 0.02	–
Cellulase activity (AU)	2.1 ± 0.01	–

Solubilization efficiency (SE) = Halo zone/diameter of bacterial colony.
Activity unit (AU) = Diameter of reaction zone/diameter of bacterial colony.
++ Medium production, + Low production, - Nil.
[a] = Values are means of three replications ± standard error.

Table 3
Type of antimicrobial peptide biosynthetic genes present in shortlisted PGPR isolates.

Sl No.	Isolate code	PGPR	Antimicrobial peptide detected
1	IISRTB1	*Bacillus pumilus*	Iturin, Surfactin
2	IISRTB2	*Bacillus cereus*	Iturin, Surfactin
3	IISRTB4	*Bacillus safensis*	Bacillomycin D, Iturin, Surfactin
4	IISRTB5	*Pseudomonas* spp	Diacetyl phloroglucinol
5	IISRTB7	*Pseudomonas aeruginosa*	Pyrrolnitrin, pyoluteorin, diacetyl phloroglucinol
6	IISRGB2	*Bacillus cereus*	Fengycin
7	IISRGB3	*Phytobacter diazotrophicus*	Bacillomycin D, fengycin
8	IISRGB7 (3)	*Bacillus cereus*	Bacillomycin D,fengycin

773 bp fragment, respectively from *Pseudomonas* spp (IISR TB5). However, none of the shortlisted PGPR strains showed amplification for CelBF and CelBR primers and PM 2 and PM7-26R primers specific to cellulase and HCN genes, respectively.

Table 4a
Effect of PGPR and chemical treatments on sprouting, yellowing of tillers, soft rot incidence, and rhizome yield of turmeric in the green house.

	Sprouting (%)	Rhizome yield (g pot^{-1})	Percent disease index	
			Yellowing of tillers	Soft rot/rhizome rot
T1	91.5 (73.11)^{a}	286.8^{b}	8.88 (17.31)^{a}	14.04 (21.97)^{a}
T2	84.48 (67.13)^{b}	246.23^{c}	11.41(19.71)^{c}	22.05(27.98)^{c}
T3	93.5 (75.31)^{a}	297.33^{a}	7.78 (16.18)^{a}	12.68 (20.79)^{a}
T4	77.2 (61.48)^{c}	152.93^{d}	31.48 (34.11)^{b}	51.17 (45.67)^{b}
T5	66.6 (54.72)^{d}	129.53^{e}	92.46 (74.24)^{d}	91.24 (72.98)^{d}
T6	64.3 (53.4)^{e}	137.0^{e}	–	–

Figures in parentheses are Arc Sine transformed values.
In a column, means followed by the same letter are not significantly different at $P < 0.05$.
T1-IISR TB4 (*Bacillus safensis*) - rhizome treatment, spraying and drenching 30, 60 and 90 DAP, T2-IISR GB7 (3) (*B. cereus*) - rhizome treatment, spraying and drenching 30, 60 and 90 DAP, T3-Combined application of IISR GB7 (3) & IISR TB4- rhizome treatment, spraying and drenching 30, 60 and 90 DAP, T4-Fungicide metalaxyl-mancozeb (0.125%) as rhizome treatment and soil drench, T5-Untreated rhizomes (inoculated with pathogen), T6-Absolute control (without pathogen inoculation).

3.4. Green house evaluation of promising PGPR strains for disease suppression in turmeric

The results (Table 4a; Fig. S4a) indicated significantly ($P < 0.05$) higher sprouting in treatments with PGPR (93.5% with *B. safensis* + *B. cereus*, 91.5% with *B. safensis* alone, and 84.48% with *B. cereus* alone) compared to the chemical method (79.99%). Likewise, rhizome yield was markedly higher in all treatments with PGPR compared to the chemical treatment (Table 4a; Fig. S4a). In the treatments where *B. safensis* and *B. cereus* were applied together, rhizome yield was greater by 56.43% relative to chemical treatment, while in treatment with *B. safensis* alone and *B. cereus* alone it was greater by 54.83% and 47.39%, respectively, compared to the control. Nevertheless, the yield levels among combined PGPR treatment and *B. safensis* alone were statistically at par but significantly ($P < 0.05$) higher than sole application of *B. cereus*.

Similar to their effects *in vitro*, both the PGPR exhibited significant ($P < 0.05$) suppression of *Pythium* spp compared to the chemical method (Table 4b; Fig. S4b). In case of *P. myriotylum*, the PDI was 91.24 in the control and 51.17 in the treatment with metalaxyl-mancozeb (chemical

Table 4b
Effect of PGPR and chemical treatments on sprouting, foliar disease incidence and rhizome yield of turmeric in the green house.

	Sprouting (%)	Rhizome yield (g pot^{-1})	Foliar disease incidence (%)
T1	92.0 (73.66)[a]	297.83[a]	9.20 (17.63)[a]
T2	86.83 (68.79)[b]	249.b3[b]	11.81 (20.07)[b]
T3	94.0 (75.85)[a]	301.33[a]	8.01 (16.42)[a]
T4	72.5 (58.47)[c]	150.5[c]	25.33 (30.17)[c]
T5	64.17(53.24)[d]	127.0[d]	36.86 (37.37)[d]
T6	65.0 (53.75)[d]	134.83[d]	12.47 (20.66)[d]

Figures in parentheses are Arc Sine transformed values.
In a column, means followed by the same letter are not significantly different at $P < 0.05$.
T1-IISR TB4 (*Bacillus safensis*) - rhizome treatment, spraying and drenching 30, 60 and 90 DAP, T2-IISR GB7 (3) (*B. cereus*) - rhizome treatment, spraying and drenching 30, 60 and 90 DAP, T3-Combined application of IISR GB7 (3) & IISR TB4- rhizome treatment, spraying and drenching 30, 60 and 90 DAP, T4-Fungicide carbendazim -mancozeb (0.1%) as rhizome treatment and foliar spray, T5-Untreated rhizomes (inoculated with pathogen), T6-Absolute control (without pathogen inoculation).

treatment), which decreased significantly to 14.04 (*B. safensis* alone), 12.68 (*B. safensis* + *B. cereus*) and 22.05 (*B. cereus* alone). A similar trend was observed in case of yellowing of tillers. In the second set of experiment challenge inoculated with *Colletotrichum* spp, foliar disease incidence (PDI) decreased markedly from 36.86 in control and 25.33 in chemical treatment to 11.81 (*B. cereus* alone), 9.20 (*B. safensis*) and 8.01 (*B. safensis* + *B. cereus*). Though variations in PDI among the two PGPR isolates were statistically non-significant ($P < 0.05$), the results indicated marked suppression of all the three pathogens compared to the chemical method.

4. Discussion

In our study, majority of the shortlisted isolates produced IAA and maximum production was observed for *B. safensis* and *B. cereus* respectively. PGPR with N fixing or NH_3 producing traits are considered advantageous to crop growth and enhancement in agronomic yields (Nutaratat et al., 2014; Fu et al., 2016). Among the shortlisted isolates, 13 were found to be positive for NH_3 production. In this study, the PGPR isolates also showed the production of siderophores and HCN that are antifungal (Kotasthane et al., 2017; Michelsen and Stougaard, 2012) and also synthesized cell-wall degrading enzymes (pectinase, protease, cellulase) that can cause severe fungal cell wall aberrations (Jošić et al., 2015).

Different isolates of PGPR exhibit mechanisms like antibiosis, induction of defense responses in the host plant and competition for nutrient sources and space. Among these, antibiosis mediated by production AMPs is considered as one of the major mechanisms (Ongena and Jacques, 2008). In this study, we were able to detect the genes encoding AMPs in promising strains *B. safensis* (TB4) and *B. cereus* [IISR GB7 (3). The shortlisted strains showed the presence of atleast one of the cyclic lipopeptides such as fengycin, iturin, bacillomycin, and surfactin. These compounds play a major role in the biocontrol of several plant diseases and are also characterized by a wide antimicrobial spectrum and intense surfactant activities. The shortlisted strains showed the presence of multiple antibiotic production capability with maximum number of AMP markers iturin, bacillomycin, and surfactin were detected in *B. safensis* (TB4), while *B. cereus* [IISR GB7 (3)] showed the presence of bacillomycin and fengycin. According to Mora et al. (2011) the most common AMP gene markers were surfactin, bacillomycin, fengycin, and bacillysin in *Bacillus* strains, which could, in part, explain the machinations underlying the multiple modes of action *viz*., induced systemic resistance (Chowdhury et al., 2015), systemic acquired resistance (Gao et al., 2015) and antibiosis (Gao et al., 2016) of *Bacillus* spp.

The two bacterial isolates *viz*., IISR TB4 (*B. safensis*) and IISR GB7(3) (*B. cereus*) showed more than 80.0% inhibition of the test pathogens, whereas, *B. cereus* strain IISR GB7 (3) showed almost 80.0% suppression of rhizome rot and foliar pathogens. *B. cereus* is a Gram (+) bacterium commonly found in soil, albeit occupying a variety of niches like food, soil, invertebrates, plants and there are many reports that prove the benefits of *B. cereus* for phytopathogen control (Khan and Bano, 2019; Majed et al., 2016; Zhang et al., 2019). The other isolate *B. safensis*, is a Gram (+), aerobic, biosurfactant-secreting and spore-forming rod shaped chemo heterotroph, with high tolerance to salinity (Wu et al., 2019). Similar to IISR GB7 (3), this strain also exhibited remarkable degree of disease suppression with *P. aphanidermatum, C. capsici*, *M. phaseolina* and *F. oxysporum*. Compared to other *Bacillus* spp, there are only a few reports on the PGP and biocontrol abilities of *B. safensis*. Nevertheless, such marked antifungal effects can be ascribed to the presence of AMPs which indicated the ability to secrete a plethora of antimicrobial compounds causing cytolysis, membrane disruption, inhibition of mycelial growth & spore germination, synthesis of siderophores, HCN, enzymes, antibiotics etc (Ali et al., 2020). Reports on biocontrol by *B. safensis* in crops include suppression of grey mould in tomato caused by *Botrytis cinerea* (Berrada et al., 2012), wilt in squash caused by *Phytophthora capsici* (Zhang et al., 2010), blast disease in rice caused by Magnaporthe oryzae (Rong et al., 2020). The phylogenetic tree constructed using 16S rRNA sequences indicated the relationship between our strain, IISR-TB4 MT192800 (B. safensis) and other PGPR. It shared a very close relationship with other reported B. safensis strains (NCBI MT186235, MT163316, AF234854, AY030327 and KX809601) including B. pumilus strains (AY167879 and NR043242).

Both bacterial isolates *B. safensis* and *B. cereus* were selected for the greenhouse study on disease suppression and growth promotion in turmeric. We did not sterilize the soil used for the greenhouse study because a vital factor that has to be considered during the screening of PGPR is their potency in varied soil conditions/types where they are expected to be deployed (Rana et al., 2011). The results showed that both the isolates, *B. safensis* and *B. cereus* varied in their effects on sprouting, soft rot and foliar disease incidence and they performed better than the chemical treatment. In the treatments involving PGPR, soft rot incidence ranged from 12.05 to 21.30%, whereas in the control treatment, the incidence was 79.45%. In case of foliar disease incidence, PDI decreased markedly from 36.16 in control and 37.04 in chemical treatment to 12.84 (*B. cereus* alone), 10.22 (*B. safensis*) and 9.04 (*B. safensis* + *B. cereus*). Enhanced sprouting and yield indicated the superior PGP capacity of the two PGPR in terms of production of IAA, GA and NH_3. Both *B. safensis* and *B. cereus* were positive for synthesis of such PGP compounds and this was reflected on sprouting and rhizome yield. Consistent with our results, growth promotion by *B. safensis* have been registered in crops like wheat (Akinrinlola et al., 2018; Chakraborty et al., 2018), maize (Kavamura et al., 2013) and lentil (Roy et al., 2018). Also, there are several reports on plant beneficial effects of *B. cereus* in plants (Khan and Bano, 2019; Roy et al., 2018; Wang et al., 2018).

While *B. cereus* and *B. safensis* applied together were relatively better than *B. safensis* applied alone, the results were statistically non-significant. A major drawback with *B. cereus*, is that many strains cause food contamination, eye and respiratory infection (Majed et al., 2016), gastrointestinal diseases (Tirloni et al., 2019) and have been reported to cause bacteraemia in immuno compromised patients (Pedemonte et al., 2020). Therefore, the use of *B. safensis* (IISR-TB4 MT192800) alone appears to be a rational approach for ensuring a non-chemical method of multiple disease suppression in turmeric.

5. Conclusions

Both *B. cereus* [IISR GB7 (3)] and *B. safensis* (IISR-TB4) exhibited significant direct and indirect plant growth promoting traits and biocontrol in turmeric. Our greenhouse experiment results demonstrated that the soft rot incidence in turmeric treated with *B. safensis* decreased by 84.61% and 72.56% respectively, compared to fungicide

and control treatments. Similarly significant reduction in rot incidence 75.83% and 67.95% respectively was recorded in *B. cereus* treated plants also. The dominance of biosynthetic genes in these rhizobacterial strains also supports the competitive role of AMP in enhancing the fitness of these strains under normal environmental conditions. These promising strains showed the presence of biosynthetic genes that encode AMPs such as bacillomycin, fengycin, iturin and surfactin. Since there are many incriminating reports on several strains of *B. cereus* in relation to human health, we suggest the use of *B. safensis*, which exhibited multiple plant growth promoting traits and the ability to concurrently suppress rhizome rot and foliar fungal pathogens. The study provides novel information on the broad-spectrum biocontrol activity of *B. safensis* against multiple fungal pathogens infecting turmeric. Nevertheless, the next vital step should involve evaluation of *B. safensis* in multiple environments for developing disease management strategies that are committed to reduced usage of fungicides in turmeric.

Availability of data and material

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Code availability

Not applicable.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All authors agree to publish the work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2022.100515.

References

Akinrinlola, R.J., Yuen, G.Y., Drijber, R.A., Adesemoye, A.O., 2018. Evaluation of Bacillus strains for plant growth promotion and predictability of efficacy by in vitro physiological traits. Internet J. Microbiol. 5686874 https://doi.org/10.1155/2018/5686874.

Ali, S., Hameed, S., Shahid, M., Iqbal, M., Lazarovits, G., Imran, A., 2020. Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. Microbiol. Res. 232, 126389. https://doi.org/10.1016/j.micres.2019.126389.

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A., Hallmann, J., 2005. Endophytic and ectophytic potato associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol. Ecol. 51, 215–229. https://doi.org/10.1016/j.femsec.2004.08.006.

Berrada, I., Benkhemmar, O., Swings, J., Bendaou, N., Amar, M., 2012. Selection of halophilic bacteria for biological control of tomato gray mould caused by *Botrytis cinerea*. Phytopathol. Mediterr. 51, 625–630. https://www.jstor.org/stable/43872349.

Cappuccino, J.G., Sherman, N., 1992. Biochemical Activities of Microorganisms, Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co, California, USA.

Chakraborty, U., Chakraborty, B., Dey, P., Chakraborty, A., 2018. *Bacillus safensis* from wheat rhizosphere promotes growth and ameliorates salinity stress in wheat. Indian J. Biotechnol. 17, 466–479. http://nopr.niscair.res.in/handle/123456789/45279.

Chowdhury, S.P., Hartmann, A., Gao, X., Borriss, R., 2015. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42-a review. Front. Microbiol. 6, 780. https://doi.org/10.3389/fmicb.2015.00780.

Deleu, M., Paquot, M., Nylander, T., 2008. Effect of fengycin, a lipopeptide produced by Bacillus subtilis, on model biomembranes. Biophys. J. 94 (7), 2667–2679. https://doi.org/10.1529/biophysj.107.114090.

Dinesh, R., Anandaraj, M., Kumar, A., Bini, Y.K., Subila, K.P., Aravind, R., 2015. Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. Microbiol. Res. 173, 34–43. https://doi.org/10.1016/j.micres.2015.01.014.

Fu, S.F., Sun, P.F., Lu, H.Y., Wei, J.Y., Xiao, H.S., Fang, W.T., Cheng, B.Y., Chou, J.Y., 2016. Plant growth-promoting traits of yeasts isolated from the phyllosphere and rhizosphere of *Drosera spatulata* Lab. Fungal Biol 12, 433–448. https://doi.org/10.1016/j.funbio.2015.12.006.

Gao, S., Wu, H., Yu, X., Qian, L., Gao, X., 2016. Swarming motility plays the major role in migration during tomato root colonization by *Bacillus subtilis* SWR01. Biol. Control 98, 11–17. https://doi.org/10.1016/j.biocontrol.2016.03.011.

Gao, T., Foulston, L., Chai, Y., Wang, Q., Losick, R., 2015. Alternative modes of biofilm formation by plant-associated *Bacillus cereus*. Microbiol. 4, 452–464. https://doi.org/10.1002/mbo3.251.

Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.-S., Patra, J.K., 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol. Res. 206, 131–140. https://doi.org/10.1016/j.micres.2017.08.016.

Grobelak, A., Kokot, P., Hutchison, D., Grosser, A., Kacprzak, M., 2018. Plant growth-promoting rhizobacteria as an alternative to mineral fertilizers in assisted bioremediation-sustainable land and waste management. J. Environ. Manag. 227, 1–9. https://doi.org/10.1016/j.jenvman.2018.08.075.

Holt, J.G., Kreig, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. Bergey's Manual of Determinative Bacteriology, ninth ed. Williams & Wilkins, Baltimore.

Hu, X.F., Chen, J., Guo, J.F., 2006. Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World J MicrobBiotechnol 22, 983–990. https://doi.org/10.1007/s11274-006-9144-2.

Jošić, D., Ćirić, A., Soković, M., Stanojković-Sebić, A., Pivić, R., Lepšanović, Z., Glamočlija, J., 2015. Antifungal activities of indigenous plant growth promoting *Pseudomonas* spp. from alfalfa and clover rhizosphere. Front. Life Sci. 8, 131–138. https://doi.org/10.1080/21553769.2014.998776.

Kalantari, S., Marefat, A., Naseri, B., et al., 2018. Improvement of bean yield and Fusarium root rot biocontrol using mixture of *Bacillus, Pseudomonas* and *Rhizobium*. Trop. Plant Pathol. 43, 499–505. https://doi.org/10.1007/s40858-018-0252-y.

Kavamura, V.N., Santos, S.N., Silva, J.L., Parma, M.M., Avila, L.A., Visconti, A., Zucchi, T.D., Taketani, R.G., Andreote, F.D., Melo, I.S., 2013. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiol. Res. 168, 183–191. https://doi.org/10.1016/j.micres.2012.12.002.

Khan, N., Bano, A., 2019. Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. PLoS One 14, e0222302. https://doi.org/10.1371/journal.pone.0222302.

Kloepper, J.W., Rodrigo, R.K., McLroy, J.A., Collins, D.J., 1991. Analysis of populations and physiological characterization of microorganisms in rhizospheres of plant with antagonistic properties to phytopathogenic nematodes. Plant Soil 136, 95–102. https://doi.org/10.1007/BF02465224.

Kotasthane, A.S., Agrawal, T., Zaidi, N.W., Sing, U.S., 2017. Identification of siderophore producing and cynogenic fluorescent Pseudomonas and a simple confrontation assay to identify potential bio-control agent for collar rot of chickpea. Biotech 7, 137. https://doi.org/10.1007/s13205-017-0761-2.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Li, H., Li, H.B., Bai, Y., Wang, J., Nie, M., Li, B., et al., 2011. The use of *Pseudomonas fluorescens* P13 to control *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*) of oilseed rape. J. Microbiol. 49, 884–889. https://doi.org/10.1007/s12275-011-1261-4.

Liu, K., Newman, M., McInroy, J.A., Hu, C.H., Kloepper, J.W., 2017. Selection and assessment of Plant Growth-Promoting Rhizobacteria for biological control of multiple plant diseases. Phytopathology 107 (8), 928–936. https://doi.org/10.1094/PHYTO-02-17-0051-R.

Majed, R., Faille, C., Kallassy, M., Gohar, M., 2016. *Bacillus cereus* biofilms—same, only different. Front. Microbiol. 7, 1054. https://doi.org/10.3389/fmicb.2016.01054.

Meena, R.S., Kumar, S., Bohra, J.S., Jat, M.L. (Eds.), 2019. Sustainable Management of Soil and Environment. e-book, Springer, p. 411. https://doi.org/10.1007/978-981-13-8832-3.

Michelsen, C.F., Stougaard, P., 2012. Hydrogen cyanide synthesis and antifungal activity of the biocontrol strain *Pseudomonas fluorescens* In5 from Greenland is highly dependent on growth medium. Can. J. Microbiol. 58, 381–390. https://doi.org/10.1139/w2012-004.

Mora, I., Cabrefiga, J., Montesinos, E., 2011. Antimicrobial peptide genes in *Bacillus* strains from plant environments. Int. Microbiol. 14 (4), 213–223. https://doi.org/10.2436/20.1501.01.151.

Nagargade, M., Tyagi, V., Singh, M.K., 2018. Plant growth-promoting rhizobacteria: a biological approach toward the production of sustainable agriculture. In: Meena, V. S. (Ed.), Role of Rhizospheric Microbes in Soil: Volume 1: Stress Management and Agricultural Sustainability. Springer, Singapore, pp. 205–223. https://doi.org/10.1007/978-981-10-8402-7-8.

Naseri, B., Younesi, H., 2021. Beneficial microbes in biocontrol of root rots in bean crops: a meta-analysis (1990–2020). Physiol. Mol. Plant Pathol. 116, 101712. https://doi.org/10.1016/j.pmpp.2021.101712.

Naseri, B., Hemmati, R., 2017. Bean root rot management: recommendations based on an integrated approach for plant disease control. Rhizosphere 4, 48–53. https://doi.org/10.1016/j.rhisph.2017.07.001.

Ni, K., Wang, Y., Li, D., Cai, Y., Pang, H., 2015. Characterization, identification and application of lactic acid bacteria isolated from forage paddy rice silage. PLoS One 10 (3), e0121967. https://doi.org/10.1371/journal.pone.0121967.

Nutaratat, P., Srisuk, N., Arunrattiyakorn, P., Limtong, S., 2014. Plant growth-promoting traits of epiphytic and endophytic yeasts isolated from rice and sugar cane leaves in Thailand. Fungal Biol 118, 683–694. https://doi.org/10.1016/j.funbio.2014.04.010.

Ongena, M., Jacques, P., 2008. Bacillus lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16, 115–125. https://doi.org/10.1016/j.tim.2007.12.009.

Panja, B.N., De, D.K., Majumder, D., 2001. Evaluation of turmeric (*Curcuma longa* L.) genotypes for yield and leaf blotch disease (*Taphrina maculans* Butl.) for Terai region of West Bengal. Environ. Ecol. 19, 125–129.

Pedemonte, N.B.A., Rocchetti, N.S., Villalba, J., Tenenbaum, D.L., Settecase, C.J., Bagilet, D.H., Colombo, L.G., Gregorini, E.R., 2020. *Bacillus cereus* bacteremia in a patient with an abdominal stab wound. Rev. Argent. Microbiol. 52 (2), 115–117. https://doi.org/10.1016/j.ram.2019.07.003.

Rajalakshmi, J., Durgadevi, D., Harish, S., Raguchander, T., 2016. Morphological and molecular characterization of *Pythium aphanidermatum* the incitant of rhizome rot in turmeric. Int. J. Environ. Ecol. Fam. Urban Stud. 6 (4), 1–8.

Rana, A., Saharan, B., Joshi, M., Prasanna, R., Kumar, K., Nain, L., 2011. Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann. Microbiol. 61, 893–900. https://doi.org/10.1007/s13213-011-0211-z.

Raymaekers, K., Poneta, L., Holtappels, D., Berckmans, B., Cammue, B.P.A., 2020. Screening for novel biocontrol agents applicable in plant disease management – a review. Biol. Control 1, 144. https://doi.org/10.1016/j.biocontrol.2020.104240.

Rong, S., Xua, H., Lia, L., Chena, R., Gao, X., Xu, Z., 2020. Antifungal activity of endophytic *Bacillus safensis* B21 and its potential application as a biopesticide to control rice blast. Pestic. Biochem. Physiol. 162, 69–77. https://doi.org/10.1016/j.pestbp.2019.09.003.

Roy, T., Bandopadhyay, A., Sonawane, P.J., Majumdar, S., Mahapatra, N.R., Alam, S., Das, N., 2018. Bio-effective disease control and plant growth promotion in lentil by two pesticide degrading strains of *Bacillus sp*. Biol. Control 127, 55–63. https://doi.org/10.1016/j.biocontrol.2018.08.018.

Sambrook, J.F., Russell, D., 2001. Molecular Cloning: A Laboratory Manual (3-Volume Set). Cold Spring Harbor, New York.

Sawar, M., Kremer, R.J., 1995. Determination of bacterially derived auxins using a microplate method. Lett. Appl. Microbiol. 20, 282–285. https://doi.org/10.1111/j.1472-765X.1995.tb00446.x.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56. https://doi.org/10.1016/0003-2697(87)90612-9.

Stabb, E.V., Jacobson, L.M., Handelsman, J., 1994. Zwittermicin A-producing strains of *Bacillus cereus* from diverse soils. Appl. Environ. Microbiol. 60, 4404–4412. https://doi.org/10.1128/aem.60.12.4404-4412.1994.

Tindal, B.J., Sikorski, J., Smibert, R.A., Krieg, N.R., 2007. Phenotypic characterization and principles of comparative systemic. In: Reddy, C.A., Beveridge, T.J., Breznak, J. A., Marzluf, G., Schmidt, T., Snyder, L. (Eds.), Methods of General and Molecular Microbiology, third ed. ASM Press, Washington DC, pp. 330–393.

Tirloni, E., Bernardi, C., Ghelardi, E., Celandroni, F., Cattaneo, P., Stella, S., 2019. *Bacillus cereus* in fried rice meals: natural occurrence, strain dependent growth and haemolysin (HBL) production. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 114, 108393. https://doi.org/10.1016/j.lwt.2019.108393.

Turner, S., Pryer, K.M., Miao, V.P., Palmer, J.D., 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. J. Eukaryot. Microbiol. 46, 327–338. https://doi.org/10.1111/j.1550-7408.1999.tb04612.x.

Venkatakrishnan, S.S., Sudalayandy, R.S., Savariappan, A.R., 2003. Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (ZSB) strains. Braz. J. Microbiol. 34, 121–125. https://doi.org/10.1590/S1517-83822004000100020.

Wang, S., Zheng, Y., Gu, C., He, C., Yang, M., Zhang, X., Guo, J., Zhao, H., Niu, D., 2018. *Bacillus cereus* AR156 Activates Defense Responses to *Pseudomonas syringae* pv. tomato in *Arabidopsis thaliana* similarly to flg22. Mol. Plant Microbe Interact. 31, 311–322. https://doi.org/10.1094/MPMI-10-17-0240-R.

Waqas, M., Khan, A.L., Kang, S.M., Kim, Y.H., Lee, I.J., 2014. Phytohormone producing fungal endophytes and hardwood-derived biochar interact to ameliorate heavy metal stress in soybeans. Soil Biol. Fert. Soils. 50, 1155–1167. https://doi.org/10.1007/s00374-014-0937-4.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991.

Wu, T., Xu, J., Liu, J., Guo, W.H., Li, X.B., Xia, J.B., Xie, W.J., Yao, Z.G., Zhang, Y.M., Wang, R.Q., 2019. Characterization and initial application of endophytic *Bacillus safensis* strain zy16 for improving phytoremediation of oil-contaminated saline soils. Front. Microbiol. 10, 991. https://doi.org/10.3389/fmicb.2019.00991.

Yu, G.Y., Sinclair, J.B., Hartman, G.L., Bertagnolli, B.L., 2002. Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. Soil Biol. Biochem. 34, 955–963. https://doi.org/10.1016/S0038-0717(02)00027-5.

Zaidi, S., Usmani, S., Singh, B.R., Musarrat, J., 2006. Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64, 991–997. https://doi.org/10.1016/j.chemosphere.2005.12.057.

Zhang, L.N., Wang, D.C., Hu, Q., Dai, X.Q., Xie, Y.S., Li, Q., Liu, H.M., Guo, J.H., 2019. Consortium of plant growth-promoting rhizobacteria strains suppresses sweet pepper disease by altering the rhizosphere microbiota. Front. Microbiol. 10, 1668. https://doi.org/10.3389/fmicb.2019.01668.

Zhang, S., White, T.L., Martinez, M.C., McInroy, J.A., Kloepper, J.W., Klassen, W., 2010. Evaluation of plant growth promoting rhizobacteria for control of *Phytophthora* blight on squash under greenhouse conditions. Biol. Control 53, 129–135. https://doi.org/10.1016/j.biocontrol.2009.10.015.