

HOSTED BY



ELSEVIER

Contents lists available at ScienceDirect

Journal of the Saudi Society of Agricultural Sciences

journal homepage: www.sciencedirect.com

Full Length Article

Abiotic stress tolerance and antifungal activities of rhizobacteria for the management of soil-borne pathogens

Vikram Poria^a, Prakriti Jhila^a, Sandeep Kumar^a, Pawan Kumar^a, Surender Singh^{a,*}, Anuj Rana^b, Sugitha Thankappan^c, Amit Kumar Goswami^d^a Department of Microbiology, Central University of Haryana, Mahendergarh 123031, India^b Department of Microbiology (COBS & H), CCS Haryana Agricultural University, Hisar 125004, India^c School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore 641114, India^d Division of Fruits and Horticultural Technology, Indian Agriculture Research Institute (IARI), New Delhi 110012, India

ARTICLE INFO

Keywords:

Cotton
Antagonistic agents
Multi-stress tolerance
Biocontrol
Charcoal rot
Fusarium wilt

ABSTRACT

Cotton production is negatively affected by both biotic (diseases and insects) and abiotic (high temperature, salinity, water deficit, and extreme pH) factors. Soil-borne diseases, especially wilts and rots, significantly reduce cotton yield. Thus, we aimed to isolate and identify multi-stress tolerant bacterial antagonistic agents (AGAs) against two major soil-borne pathogens, *Macrophomina phaseolina* and *Fusarium oxysporum*. A total of 132 isolates with distinct morphologies were recovered from 25 different rhizospheric soil samples of cotton. A dual culture plate and broth assay confirmed the antagonistic activity of the isolates against these phytopathogens. Four selected AGAs thrived in salt stress induced by different NaCl concentrations, up to 1.71 M, except for isolate 62, which survived up to 0.85 M. Under osmotic stress, all the AGAs were tolerant of up to -1.03 MPa. Similarly, all the AGAs were able to survive over a temperature range of 20–50 °C except for isolate 62, which survived up to 45 °C and was regarded as thermotolerant. All four AGAs were able to grow at pH values ranging from 5 to 9. AGA 18 and S46-7 survived under highly acidic conditions (pH 4). These multi-stress tolerant AGAs also exhibited different plant growth-promoting activities, such as mineral solubilization, ACC-deaminase production, and IAA production. Molecular identification revealed the following AGAs: *Bacillus siamensis* SSVP1 (18), *Bacillus halotolerans* SSVP2 (34), *Pseudomonas aeruginosa* SSVP3 (62), and *Bacillus tequilensis* SSVP4 (S46-7). AGAs with multiple stress tolerance traits can serve as potential biocontrol agents in the field to reduce pesticide consumption in cotton-growing areas.

1. Introduction

Cotton (*Gossypium* spp.) is the most widely cultivated agro-industrial crop for fiber and oil. Numerous biotic (insects, pathogens) and abiotic (drought, salt, pH, and temperature) factors have detrimental effects on crop production. Current estimates indicate that insect pests and diseases account for 15–30 % of yield losses in cotton, with this number increasing to 50 % due to direct injury and plant disease transmission (Zafar et al., 2022). Abiotic stresses induce a range of physiological, molecular, biochemical, and morphological alterations that are detrimental to plant development, yield, and viability (Tahmasebi et al., 2019). They also alter plant defense responses and directly affect plant-pest interactions (Jamali et al., 2020). Globally, approximately 45 % of agricultural land is affected by drought, and approximately 19.5 % is

affected by salt; it is expected that both of these stresses will reduce the cultivable area by more than 50 % over the next two decades (Maryum et al., 2022). Due to drought and heat stress, cotton fiber production could decrease by 34 % (Billah et al., 2021). Crops are damaged by plant diseases particularly soil-borne infections at different stages of plant development, resulting in a significant decrease in crop yield. Fungi are the most devastating soil-borne pathogens and are responsible for approximately 60 % of all crop diseases (Fernandez-San Millan et al., 2021).

Macrophomina phaseolina and *Fusarium oxysporum*, two soil-borne fungal phytopathogens, are responsible for charcoal rot and Fusarium wilt disease, respectively, in cotton (Manikandan et al., 2022). At least 500 plant species are infected by *M. phaseolina*, which results in significant yield losses in soybean, maize, sorghum, and cotton. In some

* Corresponding author at: Department of Microbiology, Central University of Haryana, Mahendergarh 123031, Haryana, India.

E-mail address: surendersingh@cuh.ac.in (S. Singh).<https://doi.org/10.1016/j.jssas.2024.05.002>

Available online 14 May 2024

1658-077X/© 2024 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

groundnut cultivars, 100 % yield loss is reported due to pre-emergence *M. phaseolina* infection (Marquez et al., 2021, Degani et al., 2023). In India, yield loss in cotton due to Fusarium wilt varies from 5 to 40 % (Sain et al., 2023). These pathogens also affect the quality of cotton fiber and yield, thus affecting the ginning process (Karademir et al., 2012, Majumder et al., 2018). Antagonistic agents (AGAs) such as *Bacillus* spp., *Streptomyces* spp., and *Pseudomonas* spp., have been documented for their biocontrol activity against several phytopathogens (Marfungah et al., 2023, Ratnaningsih et al., 2023) and are effective and sustainable alternatives to conventional pesticides for the control of these diseases (Le et al., 2022). These microbes use strategies such as the production of hydrolytic enzymes, volatile organic compounds, and antifungal compounds; the induction of the host immune response; siderophore and HCN production; and the release of different metabolites, such as lipopeptides, to control various pathogens (Kumar et al., 2022). Environmental factors such as biotic and abiotic factors, nutrient availability, temperature, and moisture also affect the efficacy of bacterial AGAs against plant diseases (Bonaterra et al., 2022). To mitigate different pathogens and abiotic factors, there is a need for the isolation of multi-stress tolerant bacteria that can effectively control pathogens under different stress conditions and enhance plant growth through various plant growth-promoting activities, such as phytohormone production, mineral solubilization, and siderophore secretion (Amna et al., 2020). Our research objectives were to isolate and characterize multi-stress tolerant bacterial AGAs from the cotton rhizosphere, test their efficacy against cotton phytopathogens in plate and broth, evaluate their different plant-growth promoting activities, and determine whether there is a correlation between the antagonistic ability of these agents in broth and plates.

2. Materials and methods

2.1. Isolation of AGAs

Samples of rhizospheric soil were collected from cotton fields across several parts of the Mahendgarh district, Haryana, India (28°20' N, 76°07' E). Appropriate dilutions (10^{-4} – 10^{-7}) were spread on Nutrient Agar (NA) (HiMedia, India) plates to obtain individual colonies and incubated at 30 °C for 24–48 h (LabTech incubator, South Korea). Colonies with different morphologies were purified on NA and stored at 4 °C (Tapia-Vázquez et al., 2020, Zhou et al., 2020).

2.2. Pathogenic cultures and antagonistic activity test

Two soil-borne fungal pathogens, *M. phaseolina* (Tassi) Goidanieh ITCC-5519 and *F. oxysporum* Schlecht ITCC-5330, which were originally isolated from cotton roots, were collected from the ITCC-IARI, New Delhi. The percentage inhibition test was performed following the methods of Zhang et al. (2020) with slight modifications. A fungal disc of size 5 mm cut from an agar plate of 5-day-old pathogenic culture was placed at the center of the Sabouraud dextrose agar (SDA) (HiMedia, India) plate. The plate was radially divided into three sections and AGA was streaked 2 cm away from the fungal disc in each section. As a control, a plate with a fungal agar plug without AGA was maintained. The plates were incubated at 25 °C until the control plate displayed full growth. Using formula (A), the percentage of growth inhibition was calculated.

$$\text{Percentage inhibition} = [(D1 - D2)/(D1 - \text{size of fungal disc})] \times 100 \quad (\text{A})$$

where D1 represents the diameter of the fungal colony growth on the control plate and D2 represents the diameter of the fungal colony growth on the test plate.

2.3. Fungal pathogen biomass estimation under antagonistic assay conditions

The ability of the isolates to suppress the fungal pathogens, *M. phaseolina* ITCC-5519 and *F. oxysporum* ITCC-5330, in broth was evaluated by measuring the dry weight of the fungal mycelia in the presence of the AGAs compared to that of control by following the methods of Tamreihao et al. (2016) with a few modifications. AGAs were cultured overnight in NB containing glass tubes at 30 °C and 170 rpm, and then, 1 mL of the resulting culture was seeded into a 50 mL flask containing 25 mL of Sabouraud dextrose broth (SDB) and a 5 mm disc of fungal pathogens. Fungal discs without AGA inoculation (pathogen control) and with only AGA isolates (bacterial control) were maintained as controls. The dual broth culture was incubated for 5 days at 30 °C and 170 rpm followed by centrifugation at 7,000 rpm for 10 min using a centrifuge (Eppendorf 5810R, Germany). After removing the supernatant, the mycelia dry weight was determined by drying the pellet at 50 °C in a hot air oven (Shivaki, India). To calculate the biomass inhibition percentage the following formula was used.

$$\text{Biomass inhibition (\%)} = [(W1 - (W2 - W3))/W1] \times 100; \quad (\text{B})$$

where W1 represents the dry mycelial weight (g) of the pathogen control, W2 is the dry mycelial weight of the pathogen and bacteria (g), and W3 is the dry weight of bacteria (g) alone.

2.4. Multi-stress tolerance abilities

Cell growth experiments were conducted to evaluate the multi-stress tolerance of AGAs under four different stress conditions (salinity, drought, temperature, and acidity/alkalinity). All the AGAs were checked for their ability to grow in nutrient broth (HiMedia, India) at various NaCl concentrations (0.17 M–1.71 M), various polyethylene glycol concentrations (PEG) 6000 (5, 10, 15, 20, 25, and 30 % w/v) to create nutrient solutions with different osmotic potentials (−0.05 MPa to −1.03 MPa), various temperatures (20, 25, 30, 35, 40, 45, and 50 °C), and various pH values (4, 5, 6, 7, 8, 9, 10, 11, and 12) for acidic and alkaline stress to assess their salt, osmotic stress, temperature, and pH tolerance abilities, respectively. After 24 h of incubation at 30 °C (except for the temperature stress evaluation) and 170 rpm, the stress tolerance was evaluated by measuring the OD at 600 nm using a BioTek Synergy H1 Multimode Reader (Agilent, USA), uninoculated media were used as blank. At a certain stress level, an OD of 0.1 was indicative of tolerance in bacterial cells (Amna et al., 2020).

2.5. Fungal biomass degradation ability of multi-stress tolerant AGAs and their effect on the cytoplasmic integrity of fungal pathogens

Fungal pathogen biomass degradation ability was checked by growing fungal pathogens in SDB under static conditions for 4 days, and then inoculating the selected multi-stress tolerant AGAs (18, 34, 62, and S46-7) followed by incubation for 3 days at 30 °C and 170 rpm. The biomass degradation percentage was calculated by using formula (B). A few drops of dual culture broth were observed after staining with lactophenol cotton blue directly under a phase contrast microscope (Olympus optical microscope, Japan) to observe the effect of the AGAs on mycelial integrity and the cell wall (Alblooshi et al., 2022).

2.6. Plant growth-promoting activities of selected multi-stress tolerant AGAs

2.6.1. Mineral solubilization

AGAs were checked for their ability to solubilize minerals such as phosphate (P), potassium (K), and zinc (Zn). For quantitative estimation of P solubilization, AGAs were inoculated in Pikovskaya broth supplemented with tricalcium phosphate (HiMedia, India) and incubated at

30 °C and 170 rpm for 5 days. The amount of solubilized P was measured spectrophotometrically (Analytik Jena SPECORD 210, Germany) by recording absorbance at 600 nm as described by Senthilkumar et al. (2021) and compared with P standard (100 mg/L–1000 mg/L KH_2PO_4).

AGAs were inoculated in Aleksandrov broth medium supplemented with potassium alumino-silicate (HiMedia, India) and incubated for 5 days at 30 °C and 170 rpm for quantitative estimation of solubilized K. The supernatant was collected after centrifugation at 5000 rpm for 10 min and the amount of K solubilized was measured using a flame photometer (Esico, India) (Sun et al., 2020).

Zn solubilization by AGAs was checked by spot inoculation on modified Pikovskaya agar plates supplemented with zinc oxide (HiMedia, India) instead of tri-calcium phosphate. The plates were incubated at 30 °C for 5 days. The presence of a clear zone around the colony indicated Zn solubilization (Kamran et al., 2017).

2.6.2. Indole-3-acetic acid (IAA) production

IAA production was checked by following the method of Gang et al. (2019). The isolates were inoculated in NB supplemented with L-tryptophan (0.15 % w/v) (HiMedia, India) and incubated for 24 h at 30 °C and 170 rpm. IAA production was checked spectrophotometrically at 536 nm and compared with a standard IAA curve (10–100 µg/ml) to calculate the concentration.

2.6.3. HCN production

HCN production was checked following the methods of Bakker and Schippers (1987). The plates containing King's B media supplemented with 4.4 g/L glycine (HiMedia, India) were streaked with each isolate, and the lid of each Petri dish was placed with Whatman no. 1 filter paper soaked in 2 % sodium carbonate and 0.5 % picric acid (HiMedia, India). The plates were sealed with parafilm and incubated for 4 days at 30 °C. The presence of a deep brown colour on the filter paper indicated the production of HCN.

2.6.4. Ammonia production

The production of ammonia was checked by inoculating isolates in tubes containing peptone water and incubating them at 30 °C and 170 rpm for seven days. The yellow to brown colour appearance after the addition of 0.5 mL of Nessler's reagent (Merck, Germany) indicated ammonia production (Bhattacharyya et al., 2020).

2.6.5. Siderophore production

The production of siderophores by the selected AGA isolates was examined using chrome azurol sulfonate (CAS) agar solid medium (Merck, Germany). The presence of a yellow to orange halo around the bacterial colony indicated siderophore production (Alexander and Zuberer, 1991).

2.6.6. 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase production

ACC-deaminase production by AGAs was checked by following the protocol of Senthilkumar et al. (2021). AGAs were grown in NB overnight and centrifuged to collect cell pellets. The pellet was washed before suspending it in DF minimal salt media supplemented with 3 mM ACC (Merck, Germany) and incubated at 30 °C and 170 rpm for 72 h. The absorbance was measured at 540 nm, and the concentration of α -ketobutyrate was determined via comparison with the standard curve (0.1–1.0 µmol/mL). The protein concentration in each sample was measured using the Bradford method.

2.7. Molecular characterization of isolates

Using 16S rRNA sequencing, four selected multi-stress tolerant AGAs (18, 34, 62, and S46-7) were identified. These isolates were cultured in NB overnight at 30 °C with shaking at 150 rpm. Using the Promega Kit (Cat no. D6005), genomic DNA was extracted and amplified using the

universal primers: 27F 5'-AGA GTT TGA TCC TGG CTC AG-3' and 1492R 5'-GGT TAC CTT GTT ACG ACT T-3'. The PCR products were subsequently sequenced by Barcode Biosciences Private Limited, Karnataka, India, according to the manufacturer's protocol. The sequences were identified using EzBioCloud (accessed on 31 May 2023) (Yoon et al., 2017) and submitted to the NCBI database. ClustalW was used for multiple sequence alignment and MEGA11 was used to construct a phylogenetic tree using the neighbour-joining method (Tamura et al., 2021).

2.8. Statistical analysis

Minitab Statistical Software and Microsoft Excel were used to carry out statistical analyses of the data at the $P = 0.05$ (95 % confidence interval) level of significance. To minimize the experimental errors and variations, complete random design was used to conduct the experiments. The effect of treatments was examined by performing one-way ANOVA with replications and treatments as random effects, followed by the Tukey test ($P < 0.05$). Different letters in the figures represent significantly different activities of different AGAs under the same conditions, as assessed by ANOVA followed by the Tukey test with $P < 0.05$.

3. Results

3.1. Isolation of AGAs

From 25 different rhizospheric soil samples, a total of 132 isolates with unique morphologies were isolated and purified. These isolates were maintained and stored on NA slants and glycerol stocks at -20 °C.

3.2. Antagonistic activity of isolates against fungal pathogens

After initial screening against *M. phaseolina* ITCC-5519 and *F. oxysporum* ITCC-5330, only 30 isolates were chosen for a percentage inhibition assay. As illustrated in Fig. 1, the antagonistic activity of the isolates was evaluated using a dual-plate assay. Fig. 1A shows that compared with the control, AGA 18 and 62 inhibited the mycelial growth of *M. phaseolina* ITCC-5519. AGA 62 also degraded the mycelia of *M. phaseolina* ITCC-5519, possibly through the secretion of several easily diffusing antifungal compounds. Similar results were observed in a dual assay against *F. oxysporum* ITCC-5330 as shown in Fig. 1B.

Out of the 30 isolates, 17 showed promising activity against both pathogens (Fig. 2A). AGAs 18, S24-7, 65, S24-11, S26-1, 62, and S46-20 inhibited *M. phaseolina* ITCC-5519 with inhibition percentages of 79.61, 77.25, 76.73, 76.21, 75.69, 75.42, and 74.38 %, respectively. Against *F. oxysporum* ITCC-5330, AGAs 62, 18, 65, S24-11, S46-7, S24-7, and S46-8 exhibited maximal inhibition rates of 79.35, 77.28, 77.28, 66.54, 65.49, 65.49, and 64.97 %, respectively.

3.3. Fungal pathogen biomass inhibition under antagonistic assay conditions

Isolate 62 showed the highest fungal biomass inhibition (99.88 %) of *M. phaseolina* ITCC-5519, whereas the highest biomass inhibition of *F. oxysporum* ITCC-5330 was shown by isolate S46-7 (100 %), followed by 62 (99.54 %). The biomass inhibition of *M. phaseolina* ITCC-5519 and *F. oxysporum* ITCC-5330 by various isolates is shown in Fig. 2B. All the isolates were able to inhibit the fungal biomass of *M. phaseolina* ITCC-5519 by more than 96 % and more than 80 % of that of *F. oxysporum* ITCC-5330, except for S24-12 (67.18 %). The ability of AGAs to inhibit biomass was greater against *M. phaseolina* ITCC-5519 than against *F. oxysporum* ITCC-5330, but for some AGAs, there was no significant difference in this activity.

The correlation between the inhibitory ability of AGAs in plate assays and their capacity to inhibit fungal biomass was determined and is depicted in Fig. 3. The correlation coefficients (r) for *M. phaseolina* ITCC-

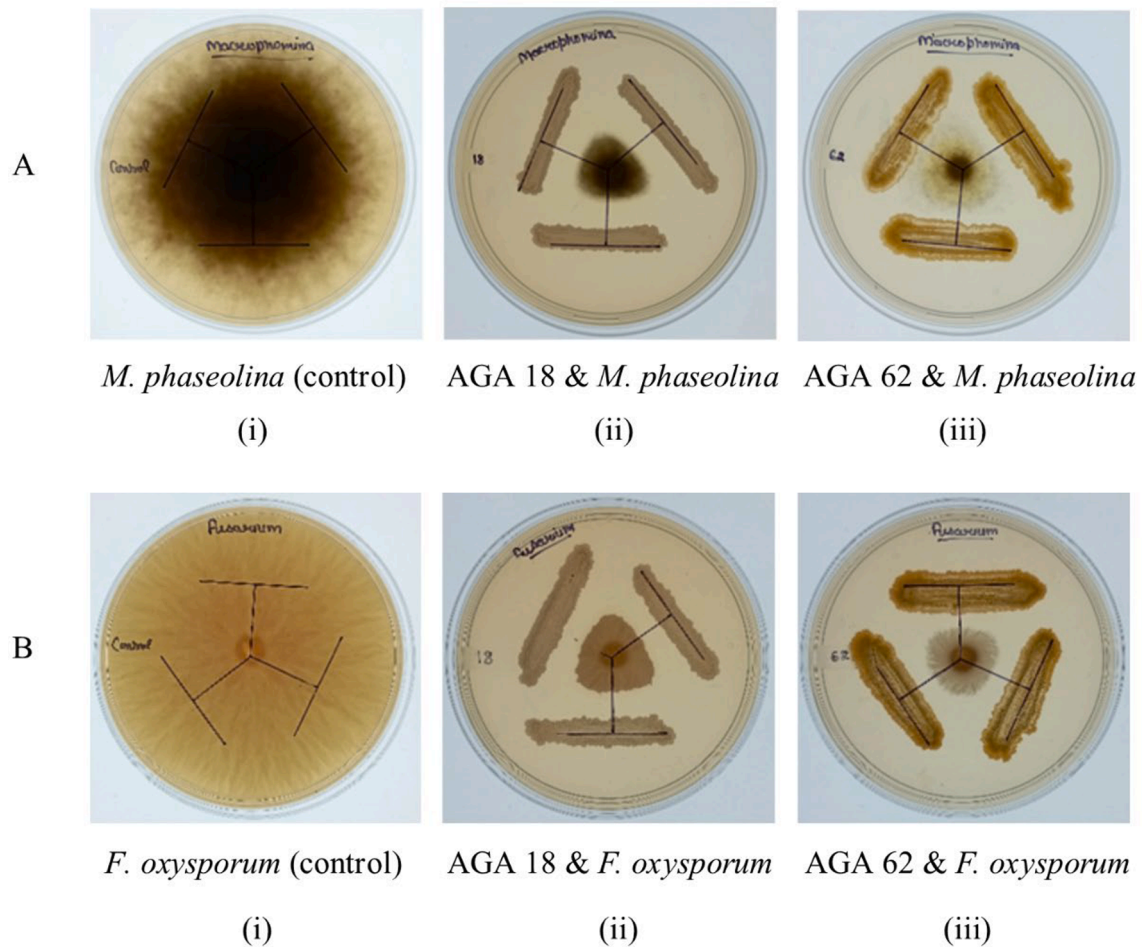


Fig. 1. Dual culture test of AGAs against pathogenic fungi; A. The antifungal activity of AGA 18 and 62 against *M. phaseolina* ITCC-5519; B. The antagonistic activity of AGA 18 and 62 against *F. oxysporum* ITCC-5330.

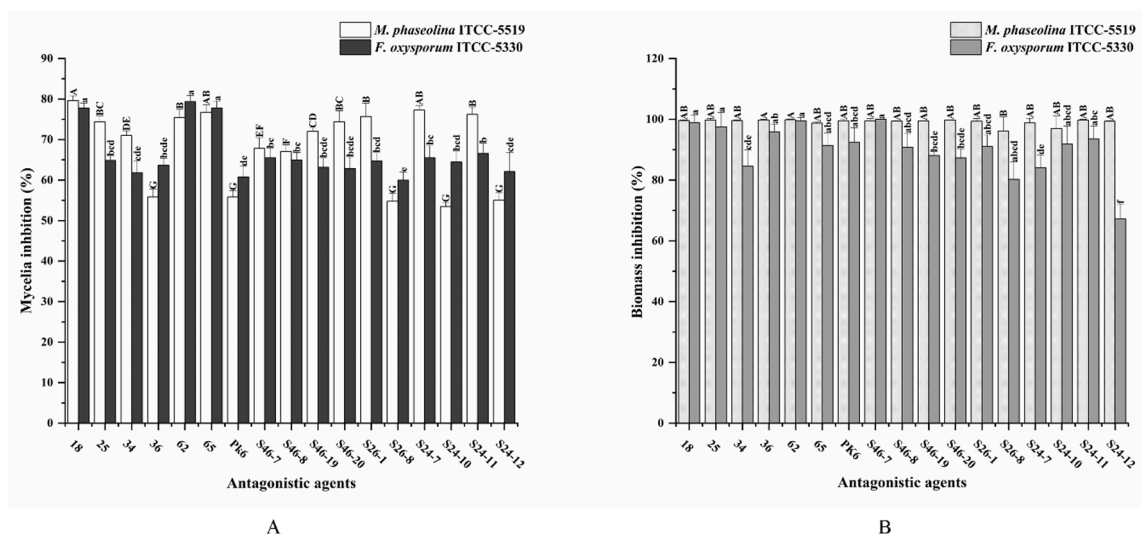


Fig. 2. Antagonistic activity of AGAs against fungal pathogens; A. Mycelial inhibition in dual plate assay; B. Biomass inhibition in broth assay.

5519 and *F. oxysporum* ITCC-5330 were 0.605 and 0.495, respectively, indicating a positive but weak relationship between the antagonistic effects of the agents on the plate and in the broth.

3.4. Multi-stress tolerance abilities

All 17 AGAs were subjected to different stress conditions, but the data for only four AGAs are shown in Fig. 4. These AGAs were selected based on their multi-stress tolerance ability and antagonistic activity

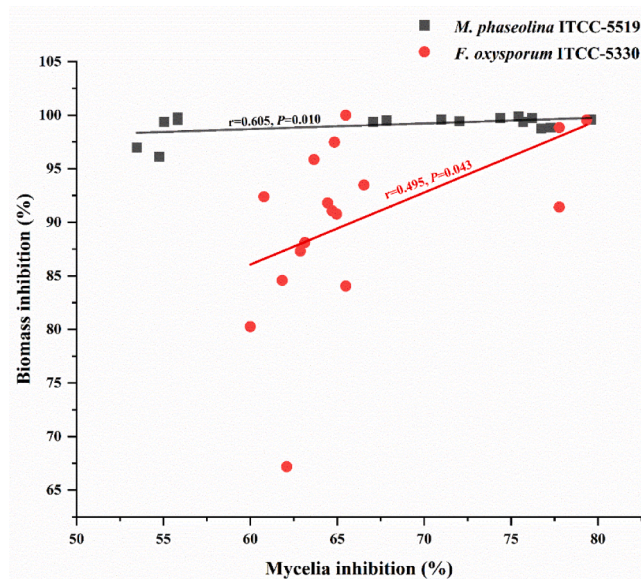


Fig. 3. Correlation between the fungal pathogen biomass inhibition ability of AGAs in broth and percentage mycelia inhibition in a plate assay.

against both pathogens. The salt tolerance of the AGAs was evaluated at various NaCl concentrations. Interestingly, the growth of the AGAs was greater in the 0.17 M NaCl treatment group than in the control group, but it decreased steadily thereafter. Three AGAs, 18, 34, and S46-7 were able to survive and tolerate salt stress up to 1.71 M NaCl, but AGA 62 showed good growth up to only 0.85 M NaCl (Fig. 4A). All the isolates were regarded as halophiles (Irshad et al., 2014).

The drought tolerance of the AGAs was evaluated with PEG 6000 at concentrations of 0 % (control), 5 % (−0.05 MPa), 10 % (−0.15 MPa), 15 % (−0.30 MPa), 20 % (−0.49 MPa), 25 % (−0.70 MPa), and 30 % (−1.03 MPa). Due to osmotic stress generated by PEG 6000, the cell OD of the AGAs decreased under stressed conditions relative to that of the control, as their growth was inhibited; nonetheless, all four AGAs (18, 34, 62, and S46-7) were regarded as tolerant even at −1.03 MPa (Fig. 4B) since their 24-hour cell-OD was more than 0.1 (Amna et al., 2020).

The AGAs were able to grow well at a temperature range of 20–50 °C, except for AGA 62, which was not able to grow at 50 °C. All four AGAs were regarded as thermotolerant due to their ability to grow at 45 °C (Fig. 4C), as an OD greater than 0.2 (600 nm) at 45 °C reflects the thermotolerant nature of microbes (Amna et al., 2020).

All four AGAs were able to grow well at pH range of 5–9. Two AGAs, 18 and S46-7, were able to thrive well even at pH 4 and were therefore regarded as moderate acidophiles (Ibrahim et al., 2023). At pH 10, AGA 34, 62, and S46-7 grew well, but only AGA 62 was able to survive at pH 11 and 12 (Fig. 4D). All these AGAs were regarded as alkaliphiles/alkali tolerant due to their ability to grow at a pH higher than 10 (Rao et al., 2022).

3.5. Fungal biomass degradation ability of multi-stress tolerant AGAs and their effect on the cytoplasmic integrity of fungal pathogens

All four multi-stress tolerant AGAs, 18, 62, 65, and S46-20, degraded the biomass of both fungal pathogens very effectively as shown in Fig. 5. The order of the biomass degradation ability of the AGAs against *M. phaseolina* ITCC-5519 was AGA 62 (97.83 %), 18 (97.59 %), S46-7 (97.20 %), and 34 (87.88 %) whereas in the case of *F. oxysporum* ITCC-5330, AGA 62 (96.81 %), S46-7 (95.37 %), 18 (85.73 %), and 34

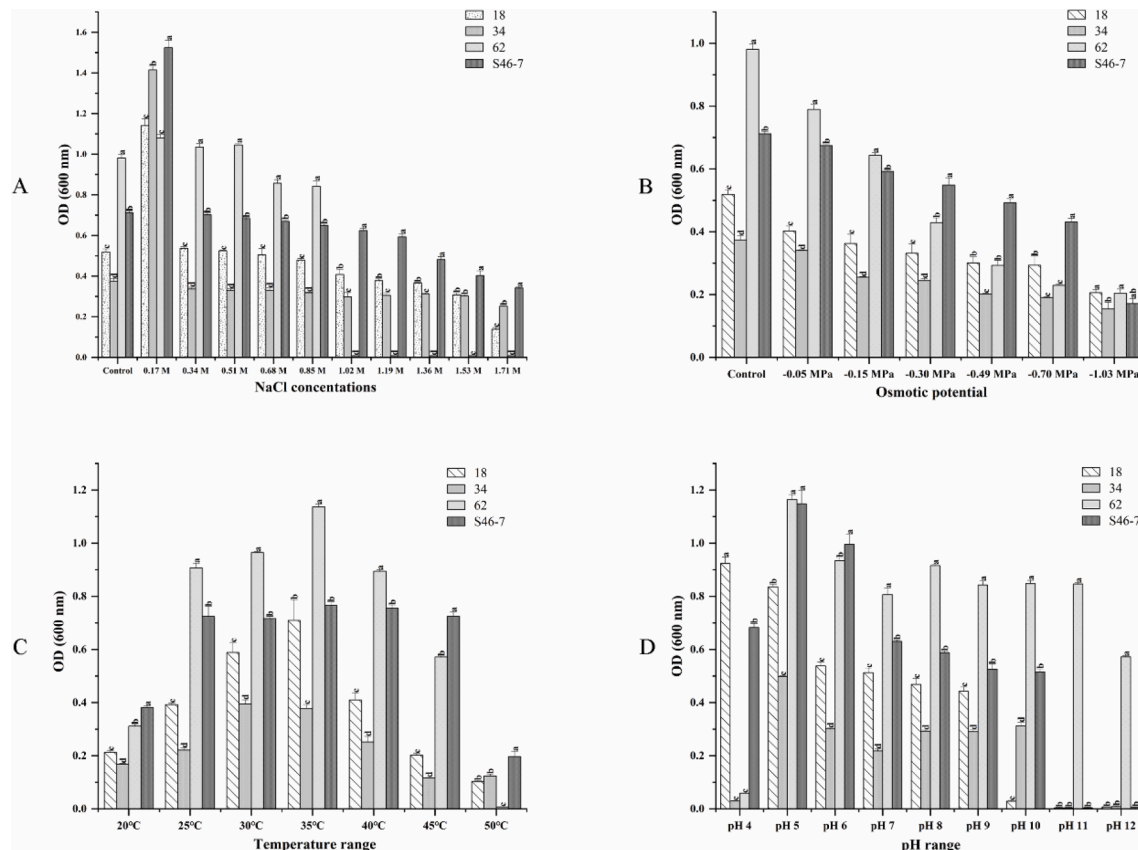


Fig. 4. Growth of different AGAs in terms of 24-hour OD under different stress conditions; A. At different NaCl concentrations; B. at different concentrations of PEG 6000; C. at different temperatures; D. at different pH values.

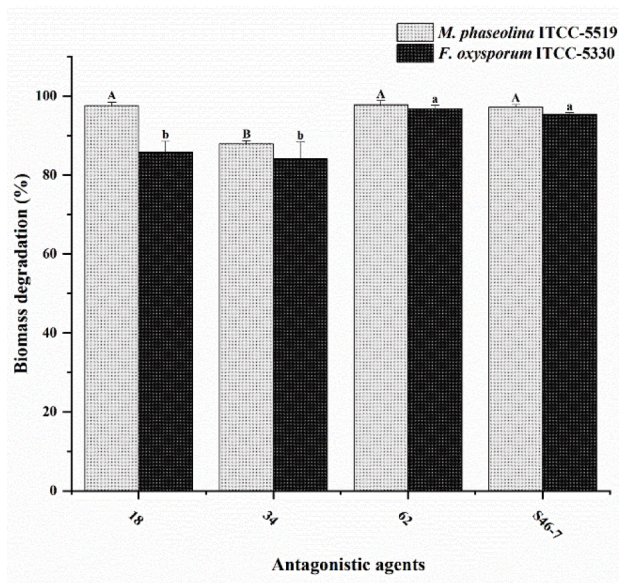


Fig. 5. Fungal pathogen biomass degradation by AGAs in broth.

(84.15 %) occurred. AGA 62 and S46-7 effectively degraded the biomass of both fungal pathogens.

Compared to that in the control group, cytoplasm breakdown was observed in the mycelia of the fungal pathogens via phase contrast microscopy (Fig. 6). In the case of the pathogen *F. oxysporum* ITCC-5330, spore development was also detected in the control; however, in the presence of AGAs, spores were either non-existent or present in a much smaller amount than in the control.

3.6. Plant growth promoting activities of selected multi-stress tolerant AGAs

All four AGAs solubilized phosphate and potassium, but only two, 18 and 62 were able to solubilize zinc. AGA 34 showed maximum phosphate and potassium solubilization of 489.17 mg/mL and 13.40 mg/mL, respectively (Fig. 7A, B). IAA production by AGAs was in the range of 4.94–11.84 µg/mL. AGA S46-7 exhibited the maximum IAA production (11.84 µg/mL) (Fig. 7C). Only AGA 62 showed ammonia, HCN, and siderophore production. For ACC-deaminase activity, all four AGAs showed positive results, and the maximum ACC-deaminase activity was shown by AGA 18 i.e., 0.37 µmol α-ketobutyrate/mg protein/min (Fig. 7D).

3.7. Molecular characterization of isolates

Based on 16S rRNA gene sequencing and identification via EzBioCloud, the identified AGAs were *Bacillus siamensis* SSV1 (18), *Bacillus halotolerans* SSV2 (34), *Pseudomonas aeruginosa* SSV3 (62), and *Bacillus tequilensis* SSV4 (S46-7). The sequences were submitted to NCBI GenBank with accession numbers of OR078455, OR078456, OR078457, and OR078458, respectively. The phylogenetic relationships of the AGAs are shown in Fig. 8.

4. Discussion

F. oxysporum and *M. phaseolina* are considered among the most devastating soil-borne pathogens of cotton, and various abiotic conditions exacerbate their infection, resulting in poor plant growth and yield (Alshoraky, 2021, Degani et al., 2022, Singh et al., 2023). Numerous studies have previously demonstrated the ability of rhizospheric microflora to control plant pathogens and enhance plant growth (Backer et al., 2018, Saeed et al., 2021). As the majority of AGAs are not stress tolerant, their antagonistic action under stressful conditions is weakened; therefore, in the present study, we isolated AGAs from the rhizosphere of cotton and evaluated their antagonistic potential against *M. phaseolina* ITCC-5519 and *F. oxysporum* ITCC-5330, as well as their efficacy under four different abiotic stresses (salt, pH, drought, and temperature). The samples were collected from Mahendergarh, India, which is a semi-arid region with very hot summers and cold winters.

The biomass inhibition and dual plate assay data suggest that these AGAs could be promising biocontrol option for controlling the tested pathogens in the field. In vitro assessment of the antagonistic activities of different AGAs has shown mycelia growth inhibition of pathogens *M. phaseolina* ITCC-5519 and *F. oxysporum* ITCC-5330. Surprisingly, *P. aeruginosa* SSV3 (62) inhibited and degraded the mycelia of *M. phaseolina* ITCC-5519 in plate assay. Various *Bacillus* and *Pseudomonas* species are reported to inhibit *Fusarium* and *Macrophomina* mycelia growth through the secretion of different antifungal compounds and secondary metabolites (Pal et al., 2001, Xu et al., 2020). Different hydrolytic enzymes, such as chitinase, glucanase, protease, and cellulase secreted by various biocontrol agents also play significant roles in the biocontrol of fungal pathogens (Jadhav et al., 2017). Several researchers have reported similar results for the mycelial growth inhibition of *Macrophomina* by *Bacillus amyloliquefaciens* (66.8 %) (Rangel-Montoya et al., 2022), *B. siamensis* (75 %) (Hussain and Khan, 2020), *Pseudomonas* sp. (61.69 %) (Sundar et al., 2021) and *Fusarium* by *B. siamensis* (47.39 %) (Khalil et al., 2021), *Streptomyces* sp. (76.54 %) (Colombo et al., 2019), and *Pseudomonas* sp. (61.69 %) (Kumar et al., 2022) in plate assays.

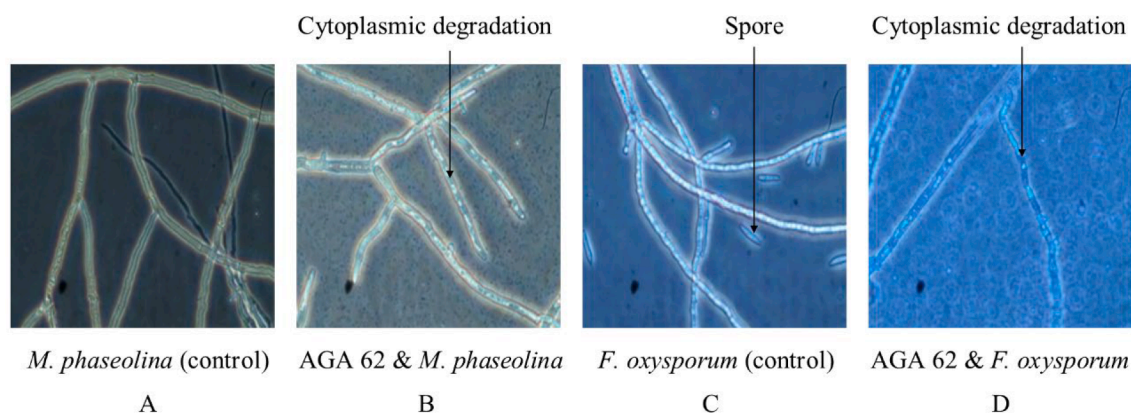


Fig. 6. Cytoplasmic degradation inside mycelia of fungal pathogens in the presence of AGAs; A. Intact mycelia of *M. phaseolina* ITCC-5519 in the control; B. Cytoplasmic degradation of *M. phaseolina* ITCC-5519 mycelia in the presence of AGA 62; C. Spores and intact mycelia of *F. oxysporum* ITCC-5330; D. Cytoplasmic degradation and no spore formation in the presence of AGA 62.

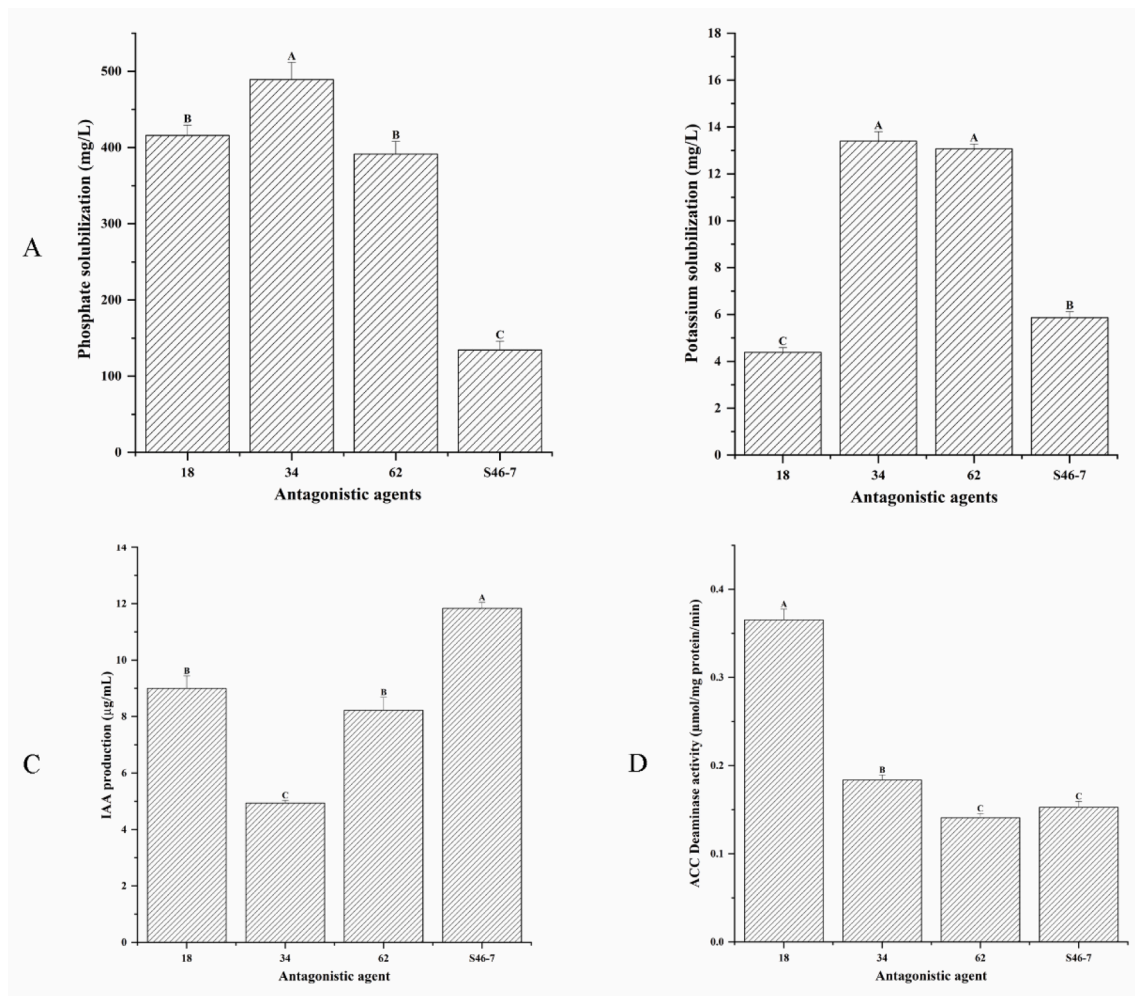


Fig. 7. Plant growth promoting activities of multi-stress tolerant AGAs; A. Phosphate solubilization activity; B. Potassium solubilization activity; C. IAA production activity; D. ACC deaminase activity.

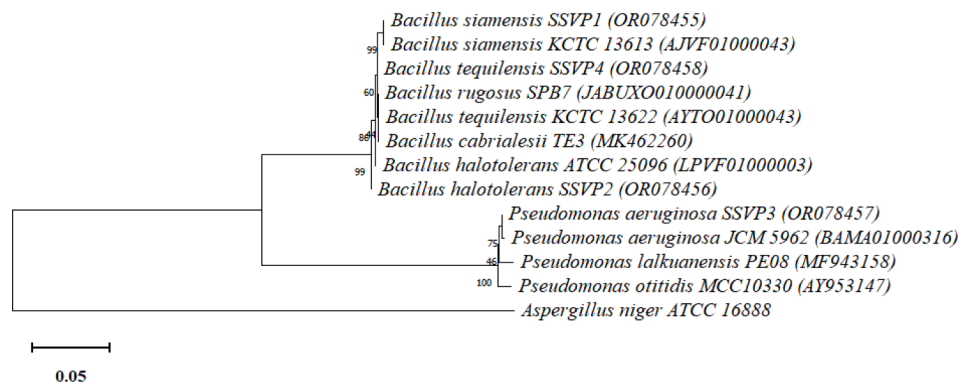


Fig. 8. Phylogenetic relationship of AGAs with other bacteria. The 16S rDNA sequences used for comparison were downloaded from EzBioCloud (<https://www.ezbiocloud.net/identify>). *Aspergillus niger* ATCC 16888 ITS region was used as an outgroup for comparison. GenBank accession numbers for all species are given in parentheses. The analysis was performed using the Bootstrap method with 1000 replications using the Kimura 2-parameter model in MEGA11 software.

The percentage biomass inhibition activity of the AGAs ranged from 96.15 to 99.88 % against *M. phaseolina* and 67.18 to 100 % against *F. oxysporum*. The antagonistic activity of these AGAs may be due to the secretion of lytic enzymes, volatile organic compounds, antifungal compounds, secondary metabolites, siderophores, and mycoparasitism (Köhl et al., 2019, Panchalingam et al., 2022). Several researchers have relied on an estimation of fungal biomass inhibition to determine the in

vitro antagonistic activity of antagonistic agents (Trivedi et al., 2008, Tamreihao et al., 2016, Mardanova et al., 2017). Trivedi et al. (2008) reported a maximum of 76.9 % biomass degradation of *Fusarium* by *Pseudomonas corrugate*. In the broth assay, greater biomass inhibition was observed than mycelial growth inhibition on the plate, possibly due to the close contact of the AGAs with fungal pathogens and the easy diffusion of antifungal metabolites secreted by the AGAs. However, a

positive correlation was observed between the ability of AGAs to inhibit mycelial growth on plates and biomass inhibition in broth, suggesting that AGAs that inhibited growth on plates should also show biomass inhibition.

Due to changing environmental conditions, crops face different abiotic stresses that reduce productivity and increase infection probability. Assessment of the stress tolerance of AGAs revealed that all four AGAs *B. siamensis* SSV1 (18), *B. halotolerans* SSV2 (34), *P. aeruginosa* SSV3 (62), and *B. tequilensis* SSV4 (S46-7) were able to tolerate multiple stresses (temperature, pH, salinity, and osmotic stress). There are several reports on the salt tolerating ability of *B. siamensis* (Gan et al., 2022), *B. halotolerans* (Zhang et al., 2018), *P. aeruginosa* (Kaleh et al., 2022), and *B. tequilensis* (Oviya et al., 2023) as well as on their drought (Zhang et al., 2018, Kushwaha et al., 2020), temperature (Lee et al., 2018), and pH (Shylla et al., 2021) tolerance ability; however, none of these reports has shown the stress tolerance ability of isolates against all four stresses. Therefore, our study is the first to report multiple stress tolerant AGAs with high antagonistic potential against the fungal pathogens *M. phaseolina* ITCC-5519 and *F. oxysporum* ITCC-5330. Bacteria use several mechanisms including exopolysaccharide secretion, biofilm formation, and osmolyte synthesis, to endure different abiotic stresses and help plants tolerate these stresses through the production of ACC deaminase, phytohormone-like auxin, gibberellin, cytokinin (CK), extracellular polymeric substances, volatile compounds, siderophores, biofilm formation, osmolytes, or increased antioxidant production (Kumar et al., 2022).

Fungal pathogens grow more slowly than antagonistic agents; therefore, to observe the effect of AGAs on fully grown mycelia of pathogens, AGAs were inoculated 4 days after fungal pathogen inoculation. This showed interesting results indicating the efficacy of the AGAs in degrading the mycelial biomass of the pathogens. The percentage biomass degradation ability of the AGAs ranged from 87.88 to 97.83 % for *M. phaseolina* and 84.15 to 96.81 % for *F. oxysporum*. The biomass degradation ability of the AGAs varied among each other as well as among pathogens, possibly due to the different biocontrol mechanisms used, varying metabolites, lytic enzyme secretion levels, and counter mechanisms of the pathogens (Gang et al., 2019, Gan et al., 2022). Under the microscope, significant morphological changes were observed in the mycelia of both pathogens compared to those of the control. Cytoplasmic degradation was visible during the co-culture of AGAs and pathogens.

AGAs exhibited different plant growth-promoting activities, such as mineral solubilization (P, K, and Zn), IAA, ACC-deaminase, ammonia, HCN, and siderophore production. These activities help plants withstand different abiotic stresses and promote plant growth. ACC-deaminase production is a well-known mechanism of stress endurance. *Bacillus* spp. and *Pseudomonas* spp. are well known for their different plant growth-promoting activities (Ali et al., 2020, Carlson et al., 2020, Marfungah et al., 2023, Ratnaningsih et al., 2023).

Multi-stress tolerant AGAs are viable alternative options for chemicals that can adapt to and withstand high-stress conditions, such as low/high temperature, osmotic stress, salt, and acidity/basic stress, such that stable populations can be maintained in stressed environments. Our study highlights the potential of multi-stress tolerant AGAs as biocontrol agents and biofertilizers due to their high antagonistic activity and different plant growth promoting activities.

5. Conclusion

The application of antagonistic agents to control soil-borne pathogens such as *M. phaseolina* and *F. oxysporum* is effective for many crops. Out of a total of 132 isolates, 30 had potential antagonistic activity against *M. phaseolina* and *F. oxysporum*. Additionally, these isolates can inhibit these pathogens under multiple stress conditions and have the potential to increase plant productivity by producing different plant growth-promoting substances. These isolates were identified as *Bacillus*

spp. and *Pseudomonas* sp. based on molecular analysis. However, a detailed study of the ability of these AGAs to establish their growth-promoting potency and biocontrol efficiency in natural environments will lead to the use of these strains as crucial bioinoculants for integrated disease management which will benefit the farmers. Therefore, pot and field studies must be done to assess and validate the true efficacy and practical implications of the findings.

Ethical statement

As this manuscript does not involve research on humans or animals, nor does it include vulnerable populations, an ethical statement is not applicable.

Funding

There was no funding received for this research.

Acknowledgments

VP acknowledges the DBT-JRF fellowship (award letter no. DBTHRDP/DBT/JRF/BET-22/1/2022-23/45) provided by the Department of Biotechnology, Government of India. The agency had no role in the study design, collection, analysis, or interpretation of data.

References

- Alblooshi, A.A., Purayil, G.P., Saeed, E.E., et al., 2022. Biocontrol potential of endophytic actinobacteria against *Fusarium solani*, the causal agent of sudden decline syndrome on date palm in the UAE. *J. Fungi* 8. <https://doi.org/10.3390/jof8010008>.
- Alexander, D.B., Zuberer, D.A., 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol. Fertil. Soils* 12, 39–45. <https://doi.org/10.1007/BF00369386>.
- Ali, S., Hameed, S., Shahid, M., et al., 2020. Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. *Microbiol. Res.* 232, 126389. <https://doi.org/10.1016/j.micres.2019.126389>.
- Alshoraky, F.S., 2021. Comparative efficacy of different chemical fungicide groups against cotton root rot. *J. Plant Protect. Pathol.* 12, 209–214. [10.21608/jppp.2021.153571](https://doi.org/10.21608/jppp.2021.153571).
- Amna, Y.X., Farooq, M.A., et al., 2020. Multi-stress tolerant PGPR *Bacillus xiamenensis* PM14 activating sugarcane (*Saccharum officinarum* L.) red rot disease resistance. *Plant Physiol. Biochem.* 151, 640–649. <https://doi.org/10.1016/j.plaphy.2020.04.016>.
- Backer, R., Rokem, J.S., Ilangumaran, G., et al., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Agriculture* 9. <https://doi.org/10.3389/fpls.2018.01473>.
- Bakker, A.W., Schippers, B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* SPP-mediated plant growth-stimulation. *Soil Biol. Biochem.* 19, 451–457. [https://doi.org/10.1016/0038-0717\(87\)90037-X](https://doi.org/10.1016/0038-0717(87)90037-X).
- Bhattacharyya, C., Banerjee, S., Acharya, U., et al., 2020. Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. *Sci. Rep.* 10, 15536. <https://doi.org/10.1038/s41598-020-72439-z>.
- Billah, M., Li, F., Yang, Z., 2021. Regulatory network of cotton genes in response to salt, drought and wilt diseases (Verticillium and Fusarium): progress and perspective. *Front. Plant Sci.* 12. <https://doi.org/10.3389/fpls.2021.759245>.
- Bonaterrea, A., Badosa, E., Daranas, N., et al., 2022. Bacteria as biological control agents of plant diseases. *Microorganisms* 10. <https://doi.org/10.3390/microorganisms10091759>.
- Carlson, R., Tugizimana, F., Steenkamp, P.A., et al., 2020. Rhizobacteria-induced systemic tolerance against drought stress in *Sorghum bicolor* (L.) Moench. *Microbiol. Res.* 232, 126388. <https://doi.org/10.1016/j.micres.2019.126388>.
- Colombo, E.M., Kunova, A., Pizzatti, C., et al., 2019. Selection of an endophytic *Streptomyces* Sp. strain DEF09 from wheat roots as a biocontrol agent against *Fusarium graminearum*. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.02356>.
- Degani, O., Becher, P., Gordani, A., 2022. Pathogenic interactions between *Macrophomina phaseolina* and *Magnaportheopsis maydis* in mutually infected cotton sprouts. *Agriculture* 12. <https://doi.org/10.3390/agriculture12020255>.
- Degani, O., Gordani, A., Dimant, E., et al., 2023. The cotton charcoal rot causal agent, *Macrophomina phaseolina*, biological and chemical control. *Front. Plant Sci.* 14. <https://doi.org/10.3389/fpls.2023.1272335>.
- Fernandez-San Millan, A., Larraya, L., Farran, I., et al., 2021. Successful biocontrol of major postharvest and soil-borne plant pathogenic fungi by antagonistic yeasts. *Biol. Control* 160, 104683. <https://doi.org/10.1016/j.biocontrol.2021.104683>.

- Gan, Y., Bai, M., Lin, X., et al., 2022. Improvement of macrolactins production by the genetic adaptation of *Bacillus siamensis* A72 to saline stress via adaptive laboratory evolution. *Microb. Cell Fact.* 21, 147. <https://doi.org/10.1186/s12934-022-01871-9>.
- Gang, S., Sharma, S., Saraf, M., et al., 2019. Analysis of indole-3-acetic acid (IAA) production in *Klebsiella* by LC-MS/MS and the Salkowski method. *Bio-Protocol* 9, e3230. <https://doi.org/10.21769/BioProtoc.3230>.
- Hussain, T., Khan, A.A., 2020. Determining the antifungal activity and characterization of *Bacillus siamensis* AMU03 against *Macrophomina phaseolina* (Tassi) Goid. *Indian Phytopathol.* 73, 507–516. <https://doi.org/10.1007/s42360-020-00239-6>.
- Ibrahim, K.S., Aishwarya, M., Kannan, R.P.B., et al., 2023. In: Chapter Nine - Secondary Metabolites from Extremophiles with Therapeutic Benefits. Recent Advances and Future Perspectives of Microbial Metabolites. Academic Press, pp. 249–267. <https://doi.org/10.1016/B978-0-323-90113-0.00011-0>.
- Irshad, A., Ahmad, I., Kim, S.B., 2014. Culturable diversity of halophilic bacteria in foreshore soils. *Braz. J. Microbiol.* 45, 563–572. <https://doi.org/10.1590/S1517-83822014005000050>.
- Jadhav, H.P., Shaikh, S.S., Sayyed, R.Z., 2017. Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: an overview. In: *Rhizotrophs: Plant Growth Promotion to Bioremediation*. S. Mehnaz. Springer Singapore, Singapore, pp. 183–203. https://doi.org/10.1007/978-981-10-4862-3_9.
- Jamali, H., Sharma, A., Roohi, et al., 2020. Biocontrol potential of *Bacillus subtilis* RH5 against sheath blight of rice caused by *Rhizoctonia solani*. *J. Basic Microbiol.* 60, 268–280. <https://doi.org/10.1002/jobm.201900347>.
- Kaleh, A.M., Singh, P., Mazumdar, P., et al., 2022. Halotolerant rhizobacteria isolated from a mangrove forest alleviate saline stress in *Musa acuminata* cv *Beengan*. *Microbiol. Res.* 265, 127176 <https://doi.org/10.1016/j.micres.2022.127176>.
- Kamran, S., Shahid, I., Baig, D.N., et al., 2017. Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. *Front. Microbiol.* 8 <https://doi.org/10.3389/fmicb.2017.02593>.
- Karademir, E., Karademir, C., Ekinci, R., et al., 2012. Effect of *Verticillium dahliae* Kleb. on cotton yield and fiber technological properties. *Int. J. Plant Prod.* 6.
- Khalil, M.M.R., Fierro-Coronado, R.A., Peñuelas-Rubio, O., et al., 2021. Rhizospheric bacteria as potential biocontrol agents against *Fusarium* wilt and crown and root rot diseases in tomato. *Saudi J. Biol. Sci.* 28, 7460–7471. <https://doi.org/10.1016/j.sjbs.2021.08.043>.
- Köhl, J., Kolnaar, R., Ravensberg, W.J., 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Front. Plant Sci.* 10 <https://doi.org/10.3389/fpls.2019.00845>.
- Kumar, S., Diksha, S.S., Sindhu, et al., 2022c. Biofertilizers: an ecofriendly technology for nutrient recycling and environmental sustainability. *Curr. Res. Microbiol. Sci.* 3, 100094 <https://doi.org/10.1016/j.crmicr.2021.100094>.
- Kumar, A., Kumar, S.P.J., Chintagunta, A.D., et al., 2022a. Biocontrol potential of *Pseudomonas stutzeri* endophyte from *Withania somnifera* (Ashwagandha) seed extract against pathogenic *Fusarium oxysporum* and *Rhizoctonia solani*. *Arch. Phytopathol. Plant Protect.* 55, 1–18. <https://doi.org/10.1080/03235408.2021.1983384>.
- Kumar, P., Singh, S., Pranaw, K., et al., 2022b. Bioinoculants as mitigators of multiple stresses: a ray of hope for agriculture in the darkness of climate change. *Heliyon* 8, e11269. <https://doi.org/10.1016/j.heliyon.2022.e11269>.
- Kushwaha, P., Kashyap, P.L., Kuppusamy, P., et al., 2020. Functional characterization of endophytic bacilli from pearl millet (*Pennisetum glaucum*) and their possible role in multiple stress tolerance. *Plant Biosyst. – Int. J. Dealing All Aspects Plant Biol.* 154, 503–514. <https://doi.org/10.1080/11263504.2019.1651773>.
- Le, K.D., Yu, N.H., Park, A.R., et al., 2022. *Streptomyces* sp. AN090126 as a biocontrol agent against bacterial and fungal plant diseases. *Microorganisms* 10 <https://doi.org/10.3390/microorganisms10040791>.
- Lee, Y.H., Jang, S.J., Han, J.-H., et al., 2018. Enhanced tolerance of Chinese cabbage seedlings mediated by *Bacillus aryabhattai* H26–2 and *B. siamensis* H30–3 against high temperature stress and fungal infections. *Plant Pathol. J.* 34, 555–566. <https://doi.org/10.5423/PPJ.OA.07.2018.0130>.
- Majumder, S., Datta, K., Sarkar, C., et al., 2018. The development of *Macrophomina phaseolina* (Fungus) resistant and glufosinate (herbicide) tolerant transgenic jute. *Front. Plant Sci.* 9 <https://doi.org/10.3389/fpls.2018.00920>.
- Manikandan, A., Jaivel, N., Johnson, I., et al., 2022. Suppression of *Macrophomina* root rot, *Fusarium* wilt and growth promotion of some pulses by antagonistic rhizobacteria. *Physiol. Mol. Plant Pathol.* 121, 101876 <https://doi.org/10.1016/j.pmp.2022.101876>.
- Mardanov, A., Fanisovna Hadieva, G., Taffkilevich Lutfullin, M., et al., 2017. *Bacillus subtilis* strains with antifungal activity against the phytopathogenic fungi. *Agric. Sci.* 8, 1–20. <https://doi.org/10.4236/as.2017.81001>.
- Marfungah, S., Puspita, F., Tjahjono, B., et al., 2023. Potential of indigenous rhizobacteria as biocontrol agents of *Xanthomonas* sp. *Philipp. J. Sci.* 152, 1539–1548. <https://doi.org/10.56899/152.05.02>.
- Marquez, N., Giachero, M.L., Declerck, S., et al., 2021. *Macrophomina phaseolina*: general characteristics of pathogenicity and methods of control. *Front. Plant Sci.* 12 <https://doi.org/10.3389/fpls.2021.634397>.
- Maryum, Z., Luqman, T., Nadeem, S., et al., 2022. An overview of salinity stress, mechanism of salinity tolerance and strategies for its management in cotton. *Front. Plant Sci.* 13 <https://doi.org/10.3389/fpls.2022.907937>.
- Oviya, G., Rangasamy, A., Ariyan, M., et al., 2023. Halotolerant nodule rhizobial and passenger endophytes alleviates salinity stress in groundnut (*Arachis hypogaea* L.). *J. Plant Growth Regul.* <https://doi.org/10.1007/s00344-023-10919-y>.
- Pal, K.K., Tilak, K.V.B.R., Saxena, A.K., et al., 2001. Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol. Res.* 156, 209–223. <https://doi.org/10.1078/0944-5013-00103>.
- Panchalingam, H., Powell, D., Adra, C., et al., 2022. Assessing the various antagonistic mechanisms of *Trichoderma* strains against the brown root rot pathogen *Pyrrhoderma noxium* infecting heritage fig trees. *J. Fungi* 8. <https://doi.org/10.3390/jof8101105>.
- Rangel-Montoya, E.A., Delgado-Ramírez, C.S., Sepúlveda, E., et al., 2022. Biocontrol of *Macrophomina phaseolina* using *Bacillus amyloliquefaciens* Strains in Cowpea (*Vigna unguiculata* L.). *Agronomy* 12. <https://doi.org/10.3390/agronomy12030676>.
- Rao, A.S., Nair, A., Nivetha, K., et al., 2022. Chapter 7 - Molecular adaptations in proteins and enzymes produced by extremophilic microorganisms. In: Arora, N.K., Agnihotri, S., Mishra, J. (Eds.), *Extremozymes and Their Industrial Applications*, Academic Press, pp. 205–230. doi: 10.1016/B978-0-323-90274-8.00002-2.
- Ratnasingh, H.R., Noviana, Z., Dewi, T.K., et al., 2023. IAA and ACC deaminase producing-bacteria isolated from the rhizosphere of pineapple plants grown under different abiotic and biotic stresses. *Heliyon* 9, e16306. <https://doi.org/10.1016/j.heliyon.2023.e16306>.
- Saeed, Q., Xiukang, W., Haider, F.U., et al., 2021. Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: a comprehensive review of effects and mechanisms. *Int. J. Mol. Sci.* 22 <https://doi.org/10.3390/ijms221910529>.
- Sain, S.K., Monga, D., Sabesh, M., et al., 2023. Diseases and disorders of cotton: a field guide for symptom based identification and management. ICAR-All India Coordinated Research Project on Cotton. ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore-641003 Tamil Nadu. 140.
- Senthilkumar, M., Amaresan, N., Sankaranarayanan, A., 2021. Estimation of ACC deaminase activity in bacterial cells. In: Senthilkumar, M., Amaresan, N., Sankaranarayanan, A. (Eds.), *Plant-Microbe Interactions: Laboratory Techniques*. Springer US, New York, NY, pp. 81–83. doi: 10.1007/978-1-0716-1080-0_18.
- Senthilkumar, M., Amaresan, N., Sankaranarayanan, A., 2021. Estimation of phosphate solubilizing capacity of microorganisms. In: Senthilkumar, M., Amaresan, N., Sankaranarayanan, A. (Eds.), *Plant-Microbe Interactions: Laboratory Techniques*, Springer US, New York, NY, pp. 59–60. doi: 10.1007/978-1-0716-1080-0_10.
- Shylla, L., Barik, S.K., Behera, M.D., et al., 2021. Impact of heavy metals on water quality and indigenous *Bacillus* spp. prevalent in rat-hole coal mines. *3 Biotech.* 11, 253. <https://doi.org/10.1007/s13205-021-02808-6>.
- Singh, B.K., Delgado-Baquerizo, M., Egidi, E., et al., 2023. Climate change impacts on plant pathogens, food security and paths forward. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-023-00900-7>.
- Sun, F., Ou, Q., Wang, N., et al., 2020. Isolation and identification of potassium-solubilizing bacteria from *Mikania micrantha* rhizospheric soil and their effect on *M. micrantha* plants. *Global Ecol. Conserv.* 23, e01141 <https://doi.org/10.1016/j.gecco.2020.e01141>.
- Sundar, H., Mohan, S.S., Sornam, A., et al., 2021. Effects of three strains of *Pseudomonas fluorescens* to soil-borne fungal pathogens and silkworm, *Bombyx mori*. *Int. J. Trop. Insect Sci.* 41, 3121–3128. <https://doi.org/10.1007/s42690-021-00506-7>.
- Tahmasebi, A., Ashrafi-Dehkordi, E., Shahriari, A.G., et al., 2019. Integrative meta-analysis of transcriptomic responses to abiotic stress in cotton. *Prog. Biophys. Mol. Biol.* 146, 112–122. <https://doi.org/10.1016/j.pbiomolbio.2019.02.005>.
- Tamreihao, K., Ningthoujam, D.S., Nimaichand, S., et al., 2016. Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. *Microbiol. Res.* 192, 260–270. <https://doi.org/10.1016/j.micres.2016.08.005>.
- Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38, 3022–3027. <https://doi.org/10.1093/molbev/msab120>.
- Tapia-Vázquez, I., Sánchez-Cruz, R., Arroyo-Domínguez, M., et al., 2020. Isolation and characterization of psychrophilic and psychrotolerant plant-growth promoting microorganisms from a high-altitude volcano crater in Mexico. *Microbiol. Res.* 232, 126394 <https://doi.org/10.1016/j.micres.2019.126394>.
- Trivedi, P., Pandey, A., Palni, L.M.S., 2008. In vitro evaluation of antagonistic properties of *Pseudomonas corrugata*. *Microbiol. Res.* 163, 329–336. <https://doi.org/10.1016/j.micres.2006.06.007>.
- Xu, W., Wang, K., Wang, H., et al., 2020. Evaluation of the biocontrol potential of *Bacillus* sp. WB against *Fusarium oxysporum* f. sp. *niveum*. *Biol. Control* 147, 104288. <https://doi.org/10.1016/j.biocontrol.2020.104288>.
- Yoon, S.-H., Ha, S.-M., Kwon, S., et al., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int. J. Syst. Evol. Microbiol.* 67, 1613–1617. <https://doi.org/10.1099/ijsem.0.001755>.
- Zafar, M.M., Mustafa, G., Shoukat, F., et al., 2022. Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton. *Sci. Rep.* 12, 10878. <https://doi.org/10.1038/s41598-022-13295-x>.
- Zhang, K., Gu, L., Zhang, Y., et al., 2020. Dinactin from a new producer, *Streptomyces badius* gz-8, and its antifungal activity against the rubber antherose fungus *Colletotrichum gloeosporioides*. *Microbiol. Res.* 240, 126548 <https://doi.org/10.1016/j.micres.2020.126548>.
- Zhang, Z., Yin, L., Li, X., et al., 2018. The complete genome sequence of *Bacillus halotolerans* ZB201702 isolated from a drought- and salt-stressed rhizosphere soil. *Microb. Pathog.* 123, 246–249. <https://doi.org/10.1016/j.micpath.2018.07.019>.
- Zhou, C., Guo, R., Ji, S., et al., 2020. Isolation of *Trichoderma* from forestry micro base and the antifungal properties of isolate TpsT17 toward *Fusarium oxysporum*. *Microbiol. Res.* 231, 126371 <https://doi.org/10.1016/j.micres.2019.126371>.