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Antifungal Potential of Non-Ribosomal Lipopeptide-Producing Indigenous Bacillus Isolates against Aspergillus flavus and their Plant Growth-Promoting Effects in Maize Crop

Maryum Syed[1]*, Farhan Aslam[2], Sumera Omrani[3], Atika Iffat[4], Aysha Arif Chahel[5], Sajjad Hussain[6], Sharoon Mirza[7], Adiba Rasool[8]

[1]* Faculty of Biological Sciences, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan. Email- maryumsyeda6@gmail.com
[2] Agriculture College, Huaian University, Huaian 223003 China
Email- aslamfarhan267@gmail.com
[3] Department of Natural and Basic Sciences, University of Turbat,
Email- sumera.omrani@uot.edu.pk[3]
[4] Institute of Biological Sciences, Universiti Malaya, Malaysia. atika.iffat11@gmail.com
[5] Affiliation: Department of Botany, Government Sadiq College Women University, Bahawalpur Email- Aysha.chahel@gscwu.edu.pk
[6] Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus, Vehari, Punjab 61100, Vehari, Pakistan sajjad7403@gmail.com
[7] City University of science and information technology.
Email- doctorsharoonmirza@gmail.com
[8] Affiliation: Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan.
ORCID: 0000-0002-0307-9856, Email- adibarasool770@gmail.com
Corresponding Author: Maryum Syed[1]*

ABSTRACT: Fungi like Aspergillus flavus and Aspergillus niger cause yield and quality loss in crop and Aspergillus flavus is the most important pathogen causing grain infection by aflatoxin. Two soil strains of Bacillus bacteria (M1 and M2) were evaluated for their antifungal activity, phosphate solubilisation capability and presence of lipopeptide biosynthetic genes and influence on maize (Zea mays L.) growth. Both strains were Gram-positive, rod-shaped, catalase-positive and oxidase-positive bacteria. In agar well diffusion assay, the cell-free supernatant of M1 exhibited inhibition zones of 35 ± 0.5 mm against A. flavus and 18 ± 0.5 mm against A. niger while M2 showed inhibition zones of 25 ± 0.5 mm against A. flavus and 20 ± 0.5 mm against A. niger. Both strains showed phosphate solubilisation index of 2.6 (M1) and 2.8 (M2) in NBRIP agar. The iturin (1113 bp), fengycin (452 bp) and bacillomycin (≈875 bp) genes were found in both strains, as well as the phosphate solubilisation pqq gene. FTIR spectra of the crude extracts revealed characteristic bands of cyclic lipopeptides (N–H/O–H ~3380 cm^{-1}, aliphatic C–H ~2928 and ~2858 cm^{-1}, ester carbonyl ~1720 cm^{-1} and amide I ~1620–1650 cm^{-1}). In pot experiment, the length of maize grain improved from 25 to 29 cm in natural soil and 32 to 36 cm in autoclaved soil and M1 produced the best wet (4 g) and dry (1.8 g) mass. A. flavus growth inhibition resulted in the reduction of plant length in natural soil and autoclaved soil to 19cm and 27cm respectively, while the plants treated with the M1 + M2 consortium in the presence of A. flavus grew to 29cm and 40cm in natural and autoclaved soil respectively. The soil autoclaved soil was 24.1-42.1% longer than the other soil. Both M1

and M2 are potential candidates for environment-friendly biocontrol and biofertiliser products towards sustainable agriculture.

1. INTRODUCTION

Maize (Zea mays L.) is important cereal crop but may be affected by fungal diseases that adversely affect grain quality and yield. Aspergillus flavus infects the crop in both pre and post-harvest and is the source of the toxic mycotoxin, aflatoxin. Both A. niger and the other pathogen result in losses of millions of dollars in the region and cause black mould in seeds, stored grain, and produce. The management of these pathogens is currently based on almost exclusive use of synthetic fungicides, which covers the soil, water and food (Mali et al., 2023). Thus, biological control (use of a living organism or its product to control a phytopathogen) is more and more studied as a more environmental-friendly alternative (Puan et al., 2023; Singh, 2024a; Salazar et al., 2023; Miljaković et al., 2020). One of the most common biocontrol agents is the Bacillus species. They are able to produce a variety of non-ribosomal lipopeptides through large non-ribosomal peptide synthetase (NRPS) complexes, colonise rhizospheres and form stress-resistant endospores (Martínez-Núñez & López, 2016; Iacovelli et al., 2021). The lipopeptides of the iturin (including bacillomycin), fengycin, and surfactin families were shown to penetrate into the fungal cell surface, leading to leakage of the cell contents (Markelova & Chumak, 2025; Meena & Kanwar, 2015; Saiyam et al., 2024; Sani et al., 2024).

Other antimicrobial metabolites and volatile compounds produced by Bacillus species include: Caulier et al., 2019; Grahovac et al., 2023; Iqbal et al., 2023; Kaspar et al., 2019. Bacillus strains and their metabolites have been proven to inhibit the growth of A. flavus on chilli, peanut and maize grains, and to decrease the production of aflatoxin (Abdel-Nasser et al., 2024; Aphaiso et al., 2024; Einloft et al., 2021; Yuan et al., 2023). Bacillomycin D from B. subtilis is found to inhibit A. flavus (Gong et al., 2014); Lipopeptides from B. velezensis are found to deform A. flavus hyphae (Li et al., 2022); Iturin A damages the cells of A. niger (Wang et al., 2024). The overproduction of mycosubtilin has been found to increase the antagonistic ability of B. subtilis (Leclère et al., 2005) and B. pumilus and B. altitudinis have both been reported as being able to control a number of fungal phytopathogens (Dobrzyński et al., 2023; Malik et al., 2024). B. velezensis HC6 and B. subtilis A3 have both demonstrated their ability to exert biocontrol over fungal diseases, particularly in maize (Liu et al., 2020; Wang et al., 2025).

In addition, many strains of Bacillus that enhance plant growth directly and that induce systemic resistance as reported by Gebreslassie et al. (2024), Jan et al. (2023), and Kloepper et al. (2004). One of the mechanisms of action is the solubilisation of phosphate (Bayisa, 2023). Phosphate-solubilizing bacteria are regularly screened on the NBRIP medium (Nautiyal, 1999), and single or mixed cultures of phosphate-solubilizing bacteria are reported to enhance the growth of maize (Massucato et al., 2022; Nkir et al., 2024). Consortia of strains with different capabilities can provide an expanded range of disease protection and enhanced plant growth (Cirillo et al., 2023; Maciag et al., 2023; Molina-Romero et al., 2021; Mortinho et al., 2022; Singh et al., 2024b).

Inoculation results vary greatly with soil conditions. Masood et al. (2025) observed that non-ribosomal peptide-producing rhizobacteria of tomato resulted in better seedling vigour and suppression of Fusarium oxysporum in autoclaved soil as compared to non-autoclaved soil. In the present study, a similar approach has been adopted which includes antagonism, PCR screening of lipopeptide biosynthetic gene clusters (Mardanova et al., 2017; Mora et al., 2015; Tapi et al., 2010) and FTIR analysis of the crude extracts (Biniarz et al., 2017; Qin et al., 2025) in both pot experiments with natural soil and autoclaved soil.

In this study, the antifungal and plant growth promoting properties of the Bacillus strains M1 and M2 were assessed. The objectives were to (i) morphologically and biochemically characterise the strains; (ii) evaluate the antifungal activity of the strains against A. flavus and A. niger; (iii) test the phosphate solubilisation ability; (iv) detect the presence of genes coding for the production of iturin, fengycin, bacillomycin and pqq and (v) characterise the crude extracts by FTIR and evaluate their effect on the growth of maize plants challenged with A. flavus in natural soil and autoclaved soil.

2. MATERIALS AND METHODS

2.1. Source of bacterial strains and fungal pathogens

Twenty-seven Bacillus strains from Quaid-i-Azam University, Islamabad (QAU) library were used which were previously uncharacterised. These strains were serially diluted and enriched in HiChrome Bacillus agar from soil. We obtained the fungal pathogens from the Plant Pathology Lab at QAU and subcultured them on Sabouraud dextrose agar (SDA).

2.2. Gram staining and colony morphology

A loopful of the 24 hour culture was taken and placed onto a drop of normal saline on a sterile glass slide and stained with crystal violet for 1 minute, Gram's iodine for 1 minute, decoloriser for 5 seconds and safranin for 1 minute and the stained slide was examined in a light microscope. Growth was recorded on nutrient agar and the following parameters on HiChrome Bacillus agar were recorded: Colony size, Colony form, Colony colour, Colony margin, Colony appearance and elevation.

2.3. Biochemical tests

Catalase was evaluated by placing 3% H2O2 on a glass slide on a 24 h culture (bubbling is positive). To test the oxidase, 24 h culture was used to filter paper impregnated with 1% tetramethyl-p-phenylenediamine (dark purple = cytochrome c oxidase). Indole was detected by streaking tryptone broth and after 24 hours incubation at 37°C, adding 10 drops of Kovacs reagent (which turns yellow to red if indole is present). Nitrate broth was used to test nitrate reduction by adding sulfanilic acid and alpha-naphthylamine (red = nitrate reductase formed nitrite). Urease was tested by preparing Urea broth (2.95 g urea in 150 mL water, the urea was added after heating) and inoculating and incubating at 37 °C for 24 h, (colour change = urease). Utilisation of citrate was also measured.

2.4. Preparation of McFarland standard and fungal inoculum

A 0.5 McFarland standard was made by adding 0.5 mL of 1.175% (w/v) BaCl2 dropwise to 85 mL of 1% (v/v) H2SO4 making up to 100 mL with 1% H2SO4 and then mixing for 3-5 minutes on a magnetic stirrer. Normal saline (0.9% w/v) was made by adding 0.9 g of NaCl to 100 mL of distilled water. The spores obtained from 7 day old cultures of SDA were resuspended in normal saline and turbidity of the suspension was adjusted to 0.5 McFarland standard.

2.5. Screening of strains against fungal pathogens

All 27 strains of fungi were screened for antifungal activity through a dual-culture assay (point inoculation) against A. niger, A. flavus, A. alternata, B. cinerea and F. oxysporum (Nysanth et al., 2022). A loopful of bacterial culture was streaked on the centre of SDA plates, which were swabbed with a spore suspension and sealed with cling film and then incubated at 30 °C. The diameters (mm) of zones of inhibition were measured at 72 h and the bacterial growth rate was assessed after 24, 48, 72, 96 and 120 h. The two strains (M1 and M2) that demonstrated the highest activity against the Aspergillus species were chosen for further studies.

2.6. Antifungal activity (agar well diffusion assay)

Both strains M1 and M2 were cultured in 200 mL of nutrient broth for 48 h in a shaking incubator at 37 °C. The cell-free supernatants were obtained by centrifuging the tubes at 10,000 rpm for 20 min. The fungal spore suspensions were plated on SDA using sterile cotton swabs. Sterile borer was used to create 6 mm diameter wells in the plates, the base of which was covered with a drop of agar. To each well 100 µL of supernatant was added and the plates were incubated at 30 °C for 72 h. The radius of inhibition was measured around the wells in mm.

2.7. Phosphate solubilisation assay

The each strain was point inoculated in the centre of NBRIP agar (Nautiyal, 1999) and phosphate solubilisation was judged by halo zone formation around the colony. Colony diameter (CD) and halo zone diameter (HZ) were measured and Phosphate Solubilisation Index (PSI) was calculated using the formula of PSI = (CD + HZ)/CD (Premono et al., 1996).

2.8. PCR detection of lipopeptide and pqq genes

The genomic DNA of strains M1 and M2 were isolated and quantified using NanoDrop and then diluted to 10-30 ng μL^{-1}. The gene specific primers for iturin, fengycin and bacillomycin synthetase genes (primers designed from the conserved

adenylation and thiolation domains of NRPS enzymes (Mora et al., 2015; Tapi et al., 2010)) and for the phosphate solubilisation pqq gene were ordered from Macrogen (Seoul, Republic of Korea) according to the supplier's instructions. We optimised the annealing temperature for each primer pair on a gradient thermal cycler (Bio-Rad). The amplification was performed by denaturation at 94 °C for 3 min, and 30 cycles of denaturation at 94 °C for 1 min, followed by annealing and extension. All PCR products were compared with 1 kb DNA ladder and separated on an agarose gel.

2.9. Extraction of lipopeptides and FTIR analysis

The strains were cultivated in mineral salt medium (MSM) and lipopeptides were extracted from the cell-free culture medium using solvents, as described by Dimkić et al., (2017). The crude extracts were analyzed by FTIR in the transmission mode using a window of 400–4000 cm^{-1} (Biniarz et al., 2017).

2.10. Pot experiment

Maize seeds were surface-sterilised with hypochlorite bleach (1 min) and then ethanol (1 min) before rinsing 3 times with distilled water. Seeds were soaked for 3 h in the proper bacterial culture and its supernatant (treatments) or distilled water (controls). The agricultural soil was collected from the Botanical Garden, QAU, Islamabad and split into two part, one autoclaved at 121 °C for 15 min (autoclaved soil) and another was used as natural soil. Pots were filled with each soil and the seeds were sown following the experimental design.

To make the inocula, each strain was grown in MSM (250 mL flasks, 37 °C, 72 h) and the supernatant was harvested by centrifugation (10,000 rpm, 15 min, 4 °C); M1 was also grown in nutrient broth for 24 h at 37 °C. A. flavus was grown in Sabouraud dextrose broth in 250 mL flasks at 30 °C and 150 rpm for one week. The pathogen culture was added in different concentrations to each pot, 5 mL of bacterial, 5 mL of supernatant and 2.5 mL of pathogen culture were added depending on the treatment. Two experimental set-ups were used. In the first, the bacterial culture and supernatant were applied at sowing and pathogen sprayed on the plants, in the second the pathogen was applied at sowing with the bacterial culture and supernatant. A total of 12 treatments were used here with each treatment performed in three replicates.

Results are given for five treatments in each soil: T1 is the control (sterilised seeds); T2 is the pathogen control (seeds inoculated with A. flavus); T3 is seeds inoculated with M1; T4 is seeds inoculated with both M1 and A. flavus; and T5 is seeds inoculated with M1, M2 and A. flavus to assess the combined effect of the two strains. Chlorophyll contents of the leaves were determined with a SPAD chlorophyll meter (Department of Plant Sciences, QAU). The length of the roots, shoots, plant, wet weight and dry weight were all measured.

2.11. Statistical analysis

Each assay was done in triplicate and the results were reported as mean ± SD. Treatment effects are reported as a % difference relative to the treatment given, that is, (treatment − reference)/reference × 100.

3. RESULTS

3.1. Gram staining and colony morphology

The Gram staining of the cells of both strains showed that they were Gram-positive cells (Table 1; Figure 1a, b). The colonies (M1) on HiChrome Bacillus agar were small, circular, flat, transparent, irregularedged with a normal appearance while colonies (M2) were small, circular, raised, blue and regularedged with a normal appearance (Figure 1c, d). Both strains were found to grow well on nutrient agar (Figure 1e, f).

Table 1: Gram staining and colony morphology of Bacillus strains M1 and M2 on HiChrome Bacillus agar

Characteristics	M1	M2
Gram staining	**Positive**	**Positive**
Cell shape	**Rod**	**Rod**
Size	**Small**	**Small**
Form	**Circular**	**Circular**

Colour	**Transparent**	**Blue**
Margin	**Irregular**	**Regular**
Appearance	**Normal**	**Normal**
Elevation	**Flat**	**Raised**

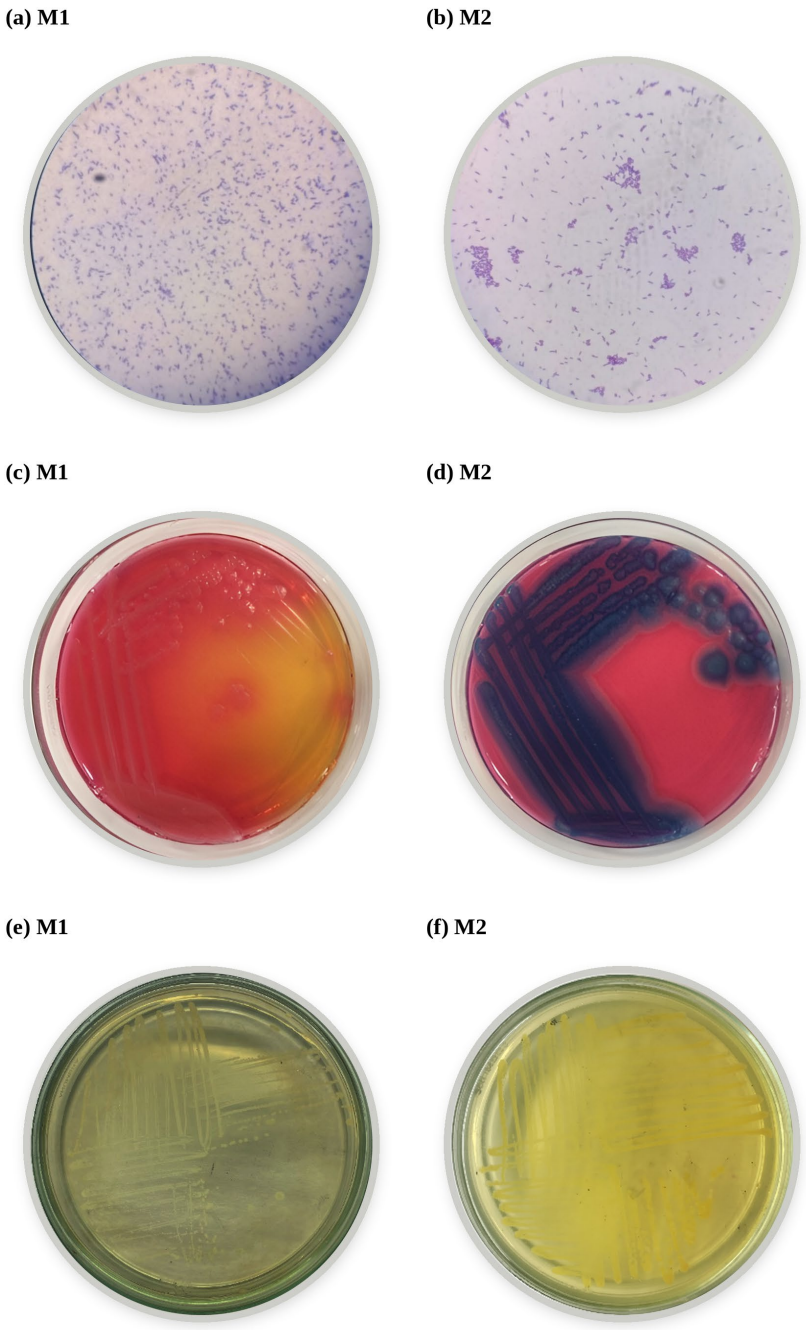

Figure 1: Gram staining (a, b), growth on HiChrome Bacillus agar (c, d), and nutrient agar (e, f) of Bacillus strains M1 and M2

3.2. Biochemical test results

Biochemical tests showed that both strains were catalase-, oxidase- and citrate-positive and were able to reduce nitrate, but neither produced indole. The two strains were only different in urease activity, being positive for M1 and negative for M2 (Table 2).

Table 2: Biochemical tests of Bacillus strains M1 and M2

Biochemical test	M1	M2
Citrate test	**+ve**	**+ve**
Urease test	**+ve**	**−ve**
Indole test	**−ve**	**−ve**
Nitrate reduction test	**+ve**	**+ve**
Catalase test	**+ve**	**+ve**
Oxidase test	**+ve**	**+ve**

3.3. Antifungal activity

Both strains showed clear inhibition zones in agar well diffusion test on SDA which confirmed the antifungal activity (Table 3; Figures 2 and 3a). The zone of inhibition produced by M1 was larger than M2 against A. flavus (35 ± 0.5 mm and 25 ± 0.5 mm respectively) but smaller and equal against A. niger (18 ± 0.5 mm and 20 ± 0.5 mm respectively).

Table 3: Zones of inhibition produced by Bacillus strains M1 and M2

Fungal pathogen	M1 (mm)	M2 (mm)
Aspergillus flavus	35 ± 0.5	25 ± 0.5
Aspergillus niger	18 ± 0.5	20 ± 0.5

Values are mean ± SD of three replicates

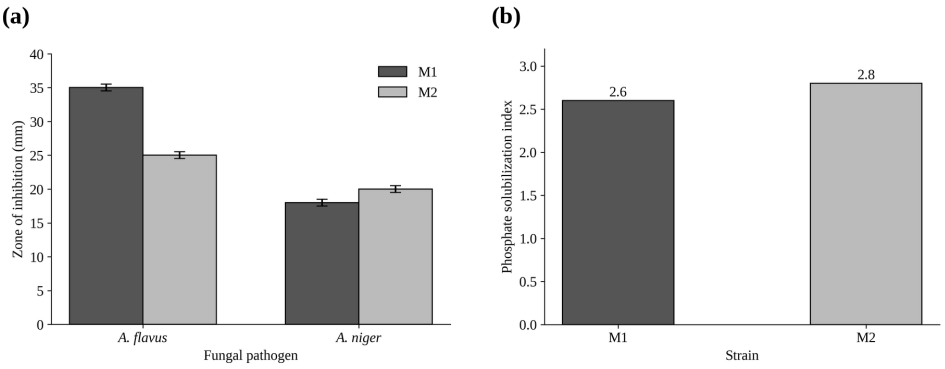

Figure 3: Zones of inhibition (a) and phosphate solubilisation index (b) of Bacillus strains M1 and M2

3.4. Phosphate solubilisation

Clear halos around the colonies were formed by both strains when growing on NBRIP agar (Figure 4). The solubilisation zone was 8 ± 0.5 mm for M1 and 9 ± 0.5 mm for M2. Colony and halo diameters were 0.7 ± 0.05 and 1.1 ± 0.05 cm for M1 (PSI 2.6) and 0.5 ± 0.05 and 0.9 ± 0.05 cm for M2 (PSI 2.8) (Table 4; Figure 3b).

Table 4: Phosphate solubilisation index of Bacillus strains M1 and M2

Strains	Colony Diameter, CD (cm)	Halo zone, HZ (cm)	PSI
M1	0.7 ± 0.05	1.1 ± 0.05	2.6
M2	0.5 ± 0.05	0.9 ± 0.05	2.8

PSI = (CD + HZ)/CD

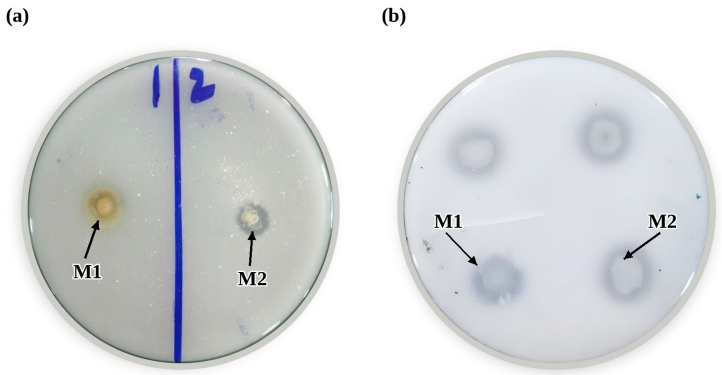

Figure 4: Phosphate solubilisation zones of M1 and M2 on NBRIP agar

3.5. Detection of lipopeptide and pqq genes

We successfully amplified fragments of all of the target genes from both strains (Table 5; Figure 5). In M1 and M2, iturin primers produced a 1113 bp product, and fengycin primers a 452 bp product (Figure 5a, b). No amplification was obtained from either strain with primers for the glyX gene, but a band of approximately 875 bp was obtained with the bacillomycin primers (Figure 5c) and a band of approximately 80 bp was obtained with the pqq primers (Figure 5d). In both strains there are genes for biocontrol and growth promotion.

Table 5: PCR detection of lipopeptide and pqq genes in Bacillus strains M1 and M2

Gene	Expected size (bp)	Observed band (bp)	M1	M2
Iturin	**1113**	**~1113**	+	+
Fengycin	**452**	**~452**	+	+
Bacillomycin	**852**	**~875**	+	+
pqq	**80**	**Band detected**	+	+

Lane L: 1 kb DNA marker; lanes 1-7: PCR products. +, distinct band observed

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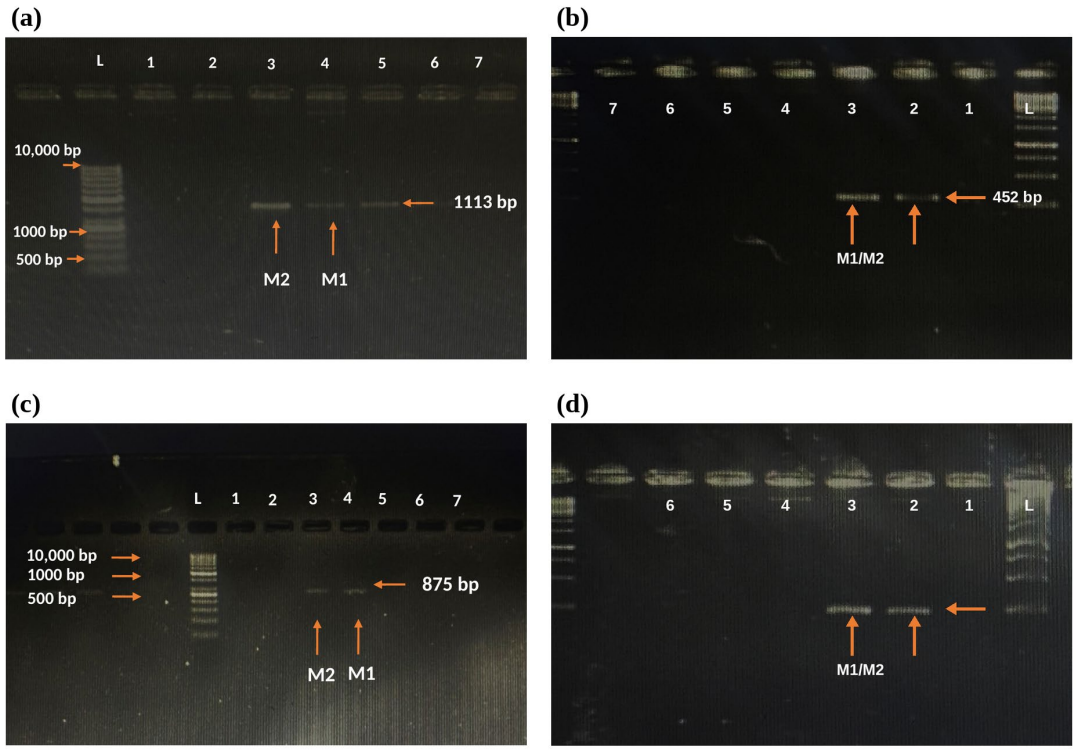

Figure 5: PCR amplification of iturin (a), fengycin (b), bacillomycin (c), and pqq (d) genes; L, 1 kb DNA ladder

3.6. FTIR analysis

The two extracts were almost alike with respect to their spectra (Table 6; Figure 6). Both exhibit broad N–H/O–H absorption around 3300–3400 cm^{-1}, strong aliphatic C–H/–SH absorption near 2928 and 2858 cm^{-1}, amide I and amide II absorptions near 1620–1650 and 1540 cm^{-1}, respectively, and an ester/lactone C=O absorption near 1720 cm^{-1}. The other absorptions at 1270, 1120 and 1040 cm^{-1} were attributed to C–O stretching and C–N stretching. All of these features suggest that lipopeptides are significant components of both extracts.

Table 6: FTIR peaks of crude extracts of Bacillus strains M1 and M2 in relation to their functional groups

M1 (cm^{-1})	M2 (cm^{-1})	Intensity	Functional group / assignment
~3380 (broad)	~3380 (broad)	Broad	N–H (amide) / O–H (hydrogen-bonded)
~2925 & ~2857	~2928 & ~2858	Medium	Aliphatic C–H stretching (CH_2, CH_3)
~1720	~1720–1725	Strong, sharp	C=O stretching (ester or lactone)
~1625–1630	~1620–1650	Medium–strong	Amide I (peptide C=O stretching)
~1540	~1535–1545	Medium	Amide II (N–H bending / C–N stretching)
~1270, 1124, 1043	~1270, 1120, 1040	Medium	C–O and C–N stretching
~900–700	~900–700	Multiple sharp	C–H out-of-plane bending, ring vibrations

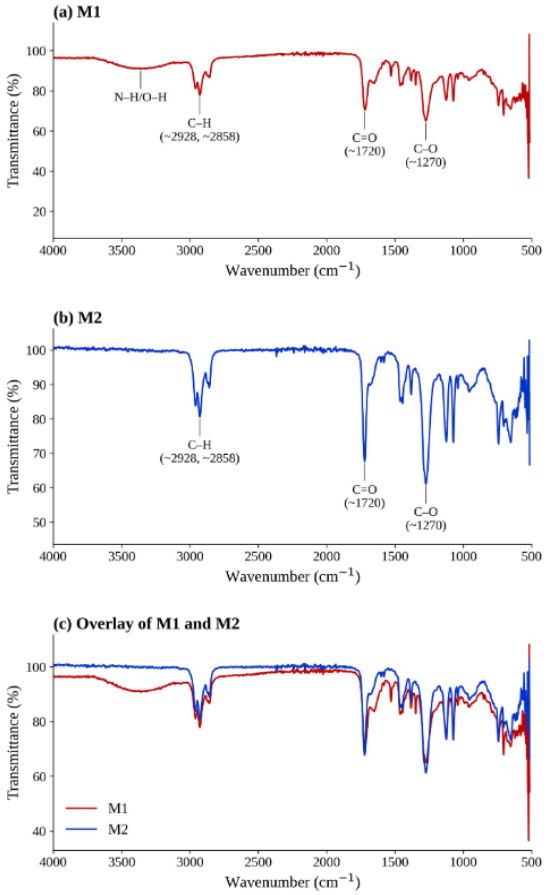

Figure 6: FTIR spectra of crude extracts of M1 (a), M2 (b) and their overlay (c)

3.7. Pot experiment

Tables 7 and 8 provide the growth parameters, and Figures 7 and 8 show the growth parameters. In natural soil, A. flavus reduced root length from 5 ± 1 to 3 ± 1 cm and shoot length from 20 ± 1 to 16 ± 1 cm, whereas M1 increased them to 7 ± 1 and 22 ± 1 cm. M1 also gave the highest wet mass (4 ± 1 g) and dry mass (1.8 ± 1 g), compared with 2 ± 1 g and 1 ± 5 g in the The wet and dry masses were 3.5 ± 1 g and 1 ± 1 g; respectively, with the pathogen; while the co-inoculated were 3.0 ± 1 g and 3.6 ± 1 g; respectively. Wet and dry mass of plants were 1 ± 1 g for all the plants (Table 7).

The consortium + A. flavus treatment yielded the longest root length (10 ± 1 cm) and shoot length (30 ± 1 cm) compared to control (7 ± 1 cm and 25 ± 1 cm) and A. flavus (6 ± 1 cm and 21 ± 1 cm) and M1 (8 ± 1 cm and 28 ± 1 cm) treatments of soil. The wet and dry mass of the plant in the co-inoculated and inoculated treatments increased from the same trend as in natural soil, highest in M1 (4 ± 1 g and 1.8 ± 1 g) and lowest in dry mass of both co-inoculated treatments (1.5 ± 1 g) (Table 8).

The same trend was observed for plant length (Table 9). In natural soil, A. flavus reduced plant length from 25 ± 1 cm in the control to 19 ± 1 cm (−24.0%), whereas M1 alone increased it to 29 ± 1 cm (+16.0%). When exposed to pathogens, plants treated with M1 grew 42.1% and 52.6% longer than the pathogen treated plants, while plants treated with the consortium grew 29 ± 1 cm. In autoclaved soil, the pathogen reduced plant length from 32 ± 1 to 27 ± 1 cm (−15.6%), and M1 alone increased it to 36 ± 1 cm (+12.5%). The plants treated with M1 + A. flavus and consortium + A. flavus grew to heights of 35 ±1 and 40 ±1 cm, respectively, which is 29.6% and 48.1% longer than the plant treated with the pathogen alone.

Plant length was also found to be higher in autoclaved soil as compared to natural soil under all treatments, by 28.0%, 24.1%, 42.1%, 29.6% and 37.9% for the control, M1, A. flavus, M1 + A. flavus and consortium + A. flavus respectively (Table 9; Figure 9).

Table 7: Plant growth parameters of maize in natural soil

Treatment	Root length (cm)	Shoot length (cm)	Wet mass (g)	Dry mass (g)
T1 Control	5 ± 1	20 ± 1	2 ± 1	1 ± 5
T2 A. flavus	3 ± 1	16 ± 1	3.5 ± 1	1 ± 1
T3 M1	7 ± 1	22 ± 1	4 ± 1	1.8 ± 1
T4 M1 + A. flavus	6 ± 1	21 ± 1	1 ± 1	1 ± 1
T5 M1 + M2 + A. flavus	6 ± 1	23 ± 1	1 ± 1	1 ± 1

Values are mean ± SD of three replicate pots

Table 8: Plant growth parameters of maize in autoclaved soil

Treatment	Root length (cm)	Shoot length (cm)	Wet mass (g)	Dry mass (g)
T1 Control	7 ± 1	25 ± 1	2 ± 1	1 ± 1
T2 A. flavus	6 ± 1	21 ± 1	3.5 ± 1	1 ± 1
T3 M1	8 ± 1	28 ± 1	4 ± 1	1.8 ± 1
T4 M1 + A. flavus	8 ± 1	29 ± 1	1 ± 1	1.5 ± 1
T5 M1 + M2 + A. flavus	10 ± 1	30 ± 1	1 ± 1	1.5 ± 1

Values are mean ± SD of three replicate pots

Table 9: Plant length of maize in natural and autoclaved soil and percentage increase in autoclaved soil

Treatment	Natural soil (cm)	Autoclaved soil (cm)	Increase in length (%)
T1 Control	25 ± 1	32 ± 1	28.0
T2 A. flavus	19 ± 1	27 ± 1	42.1
T3 M1	29 ± 1	36 ± 1	24.1
T4 M1 + A. flavus	27 ± 1	35 ± 1	29.6
T5 M1 + M2 + A. flavus	29 ± 1	40 ± 1	37.9

Values are mean ± SD of three replicate pots

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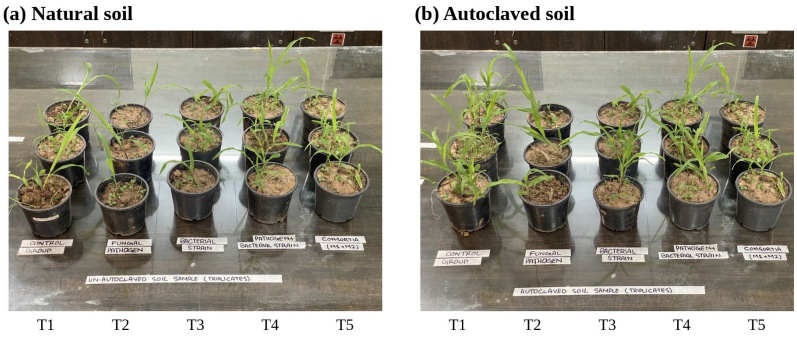

Figure 7: Pot experiment with maize in (a) natural soil and (b) autoclaved soil; T1, control; T2, A. flavus; T3, M1; T4, M1 + A. flavus; T5, M1 + M2 + A. flavus (three replicate pots per treatment)

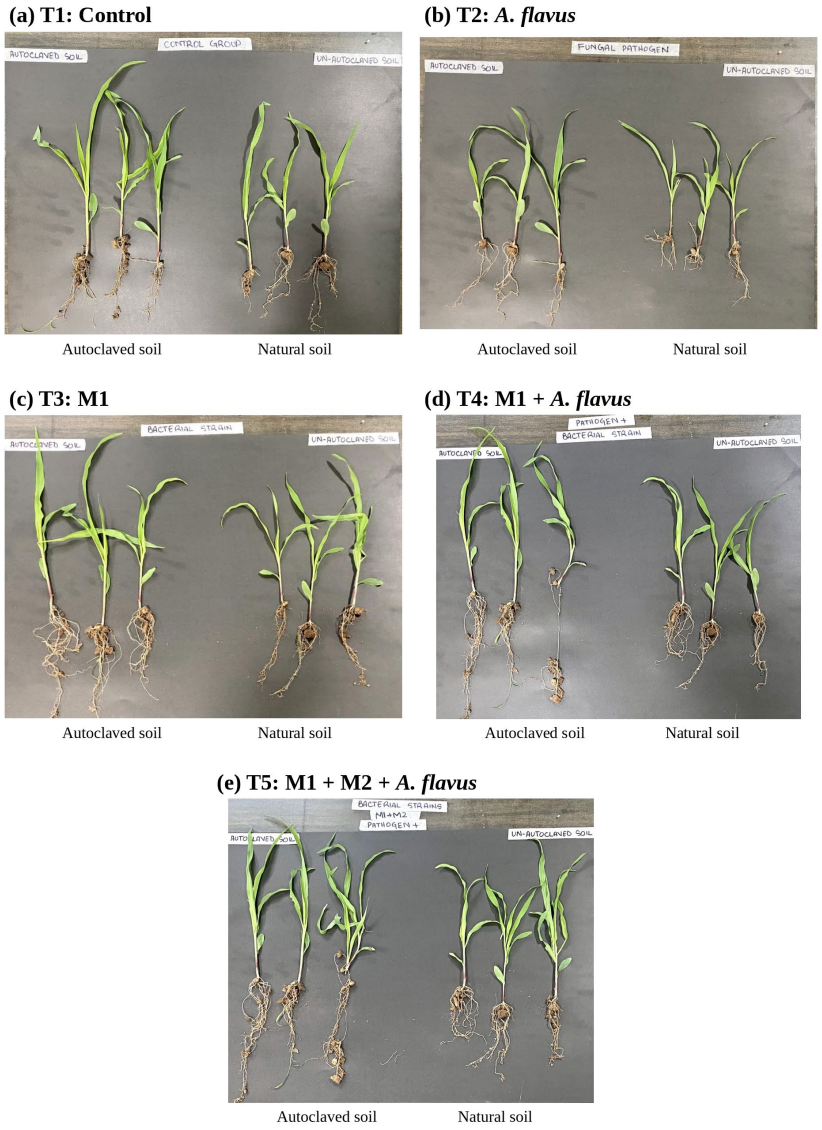

Figure 8: Maize seedlings harvested from treatments T1–T5: (a) control, (b) A. flavus, (c) M1, (d) M1 + A. flavus and (e) M1 + M2 + A. flavus; in each panel, plants from autoclaved soil (left) and natural soil (right)

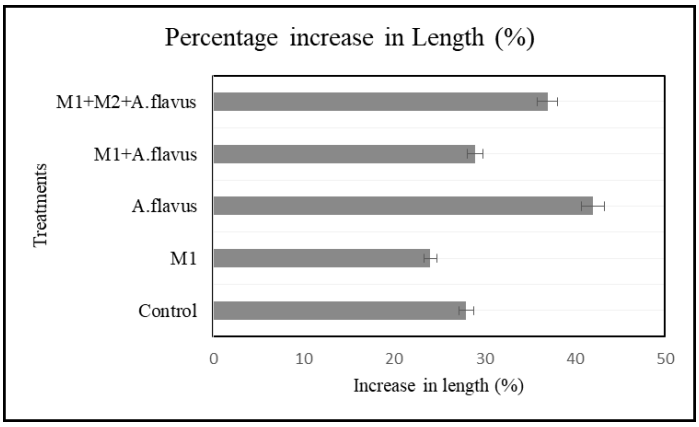

Figure 9: Percentage increase in maize length in autoclaved compared with natural soil

4. DISCUSSION

In order to control the disease sustainably, we studied two soil strains of Bacillus for their activity against A. flavus and A. niger, which cause significant losses in crops (Meena & Kanwar, 2015; Miljaković et al., 2020). Both strains were classified as Bacillus based on their biochemical characteristics (Abdel-Nasser et al., 2024; Caulier et al., 2019; Jan et al., 2023).

Inhibitions varied between the fungi targeted. When grown against A. flavus, M1 proved to be superior to M2, while against A. niger the two strains were almost indistinguishable with M2 being slightly better. These differences suggest that the kinds or amounts of antifungal products produced by each strain might be different, Such as lipopeptides or enzymes that break down the cell walls. The antagonism was also found to be dependent on the nutritional source of fungus by Masood et al., 2025 and secreted metabolites of B. sp. have been previously reported as antifungal against A. flavus (Aphaiso et al., 2024; Gong et al., 2014; Li et al., 2022) and A. niger (Dobrzyński et al., 2023; Santoso et al., 2021; Wang et al., 2024). In this case, cell-free supernatants were used in the assay and the activity can be attributed to molecules secreted into the medium and not to living cells. The impact of M1 on A. flavus indicates its potential as an antagonist of aflatoxigenic fungi and the comparable effect of both strains on A. niger suggests that the antagonism is not restricted to a single fungi species.

The medium used was the NBRIP, which suggested that both strains were able to break down the insoluble phosphate and It helps in conversion of it into soluble phosphate, which is available for plant growth (Halo formation) (Bayisa, 2023; Nautiyal, 1999). The pqq gene can be detected, giving a genetic basis for this trait: pyrroloquinoline quinone is a cofactor of the Produced enzyme: Glucose dehydrogenase which is able to convert a significant mineral phosphate solubiliser, namely gluconic acid. by bacteria. In previous research, phosphate-solubilising strains have shown to improve maize growth (Massucato et al., 2022; Nkir et al., 2024).

Iturin, fengycin and bacillomycin genes were found in both strains. The primers used were designed based on conserved NRPS motifs and PCR products were of the same size as in previous years. studies (Iacovelli et al., 2021; Martínez-Núñez & López, 2016; Mardanova et al., 2017; Mora et al., 2015; Tapi et al., 2010). These compounds, referred to as Iturin-like compounds, are thought to be the major antifungal lipopeptides of The biocontrol activity, particularly against , of the Bacillus and their production is known to increase. The case of fengycins is filamentous fungi, as seen in Markelova & Chumak (2025), Saiyam et al. (2024) and Sani et al. (2024). 2024). The crude extracts showed the characteristic absorption bands of cyclic lipopeptides such as the surfactin, fengycin and iturin. A broad band at 3259.81 cm-1 was assigned to the N–H stretching, bands at 2922.46 and 2852.62 cm-1 to Vibrational bands due to the aliphatic side chains, 1646.74 cm-1 (peptide) and 1454.91 cm-1 (methyl), were observed. For the M1 and M2 extracts, the N–H stretching was detected at 3287.67 and 3287.94 cm-1, respectively, which were referred to as aliphatic side-chain stretching. at 2932.51 and 2931.49 cm^{-1}, peptide absorption at 1640.26 and 1641.80 cm^{-1}, and methyl/aliphatic C–H absorption at 1454.56 and 1455.01 cm^{-1}. The hydrophobic fatty acid part of the peptides is matched with published lipopeptide spectra (Biniarz et al., 2017; Dimkić et al., 2017; Qin et al., 2025) in combination with the hydrophilic peptide part. However, FTIR cannot distinguish the various lipopeptide families so LC–MS must be used to identify the specific compounds.

Effect of the Bacillus strains on the pathogen was illustrated by pot experiment which showed suppression of the pathogen. A negative effect on plant growth. Bacterial inoculation resulted in a decrease in the growth inhibition of A. flavus, while M1 alone gave the highest root and The plant growth promoting effect of the rhizosphere was only observed in shoot length and highest of wet and dry mass in both soils. ability. The consortium and pathogen promoted plant growth and protected plants from pathogens as the consortium Treatment with pathogen and M1 treatments increased the treatment times relative to natural soil uninoculated. The results were corroborated by the reported biocontrol and growth promoting activity of Bacillus in maize and other crops. crops (Gebreslassie et al., 2024; Kloepper et al., 2004; Liu et al., 2020; Wang et al., 2025). The increased plant growth of the consortium over M1 when challenged by the pathogens could indicate an additive effect. of the two strains as reported for bacterial consortia (Cirillo et al., 2023; Maciag et al., 2023; Molina-Romero et al., 2021; Mortinho et al., 2022; Singh et al., 2024b)

Length of plants in autoclaved soil was greater than natural soil in all treatments, revealing less microbial competition. Growth and biocontrol were the largest increases in pathogen-treated plants, probably due to suppression of the native antagonists, and M1 + pathogen and M1 showed increases, thus supporting the growth-promoting and biocontrol activity of M1. Overall, the consortium resulted in the longest plants when challenged with pathogens and the consortium M1 provided protection and growth promotion. The same trend was observed by Masood et al. (2025) and was explained by the decrease in competition between introduced and indigenous microorganisms.

The growth stimulating effect of M1 and M2 on plants is not known. Possible contributors are production of growth-regulating substances and volatiles and activation of plant defences (Grahovac et al., 2023; Kloepper et al., 2004). Fungi that have both antifungal and growth promoting properties can be beneficial for the environment Some of the friendly disease management and yield improvement (Salazar et al., 2023; Singh et al., 2024a). Important: The pot test was performed, note that the identification of the strain is based only on its morphology and biochemistry. Once, three replicates of each treatment. Thus, the 16S rRNA gene sequencing and statistically designed trials of larger samples are recommended

5. CONCLUSIONS AND PROSPECTS

Bacillus strains M1 and M2 are good candidates for the control of A. flavus, an aflatoxin producing fungus which Heavy losses are caused in agriculture, as are A. niger. Both strains inhibited growth of fungi in vitro and M1 was a more potent inhibitor of A. flavus. In addition, they discovered iturin, fengycin and bacillomycin and pqq genes that are responsible for antifungal activity and growth promotion, Using PCR, and extracts with high levels of lipopeptides based on their FTIR spectra.

A. flavus's growth inhibiting effect, while the maize of M1 + M2 consortium improved the growth loss. plant growth. In natural and autoclaved soil. So, these strains are recommended to be field tested for crop protection and yield improvement. More studies are recommended to ensure the uniformity of spore production, shelf life and handling of the spores. The following: (1) Genome editing Technologies for stacking beneficial traits; (2) improved yield and reduced disease; (3) yield testing and validation; (4) GMO labelling; and (5) farmer involvement in the commercialization and uptake on the farmer's field. Antimicrobial activity of fungal metabolites, including CRISPER, was carried out in single organisms; (iii) chemical and genomic analysis of the antimicrobial activity of fungal metabolites. Localization of their biosynthetic gene clusters and action mechanism by metabolites and enzymes; and (iv) The incorporation of non-pathogenic fungi in the research scope, optimization of this section and testing for absence.

Infections caused by O. solani on different sources. Optimization of this section; Testing for absence of R. solani from various sources. Plackett–Burman screening was used to optimize the fermentation and formulation processes, followed by the response surface methodology. Optimization was done by Plackett–Burman screening followed by response surface methodology for the fermentation and formulation processes. surface methodology, respectively. To verify spore production and shelf-life; (vi) to verify the viability of the spores. (vi) develop regulatory, storage and application strategies to; (vii) Management and legislative aspects related to the use of the land and water resources of the region. The land and water resources of the region are used for various purposes and the legal aspects of these uses are explored.

REFERENCES

[1] Abdel-Nasser, A., Badr, A. N., Fathy, H. M., Ghareeb, M. A., Barakat, O. S., & Hathout, A. S. (2024). Antifungal, antiaflatoxigenic, and cytotoxic properties of bioactive secondary metabolites derived from Bacillus species. Scientific Reports, 14(1), 16590. https://doi.org/10.1038/s41598-024-66700-y

[2] Aphaiso, P., Mahakhan, P., & Sawaengkaew, J. (2024). Bacillus siamensis 3BS12-4 extracellular compounds as a potential biological control agent against Aspergillus flavus. Journal of Microbiology and Biotechnology, 34(8), 1671–1679. https://doi.org/10.4014/jmb.2402.02053

[3] Bayisa, R. A. (2023). Bacillus velezensis AR1 mediated plant nourishing through solubilization of hardly soluble phosphorus nutrient sources. Cogent Food & Agriculture, 9, 2276561. https://doi.org/10.1080/23311932.2023.2276561

[4] Biniarz, P., Łukaszewicz, M., & Janek, T. (2017). Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: a review. Critical Reviews in Biotechnology, 37(3), 393–410. https://doi.org/10.3109/07388551.2016.1163324

[5] Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., & Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the Bacillus subtilis group. Frontiers in Microbiology, 10, 302. https://doi.org/10.3389/fmicb.2019.00302

[6] Cirillo, V., Romano, I., Woo, S. L., Di Stasio, E., Lombardi, N., Comite, E., Pepe, O., Ventorino, V., & Maggio, A. (2023). Inoculation with a microbial consortium increases soil microbial diversity and improves agronomic traits of tomato under water and nitrogen deficiency. Frontiers in Plant Science, 14, 1304627. https://doi.org/10.3389/fpls.2023.1304627

[7] Dimkić, I., Stanković, S., Nišavić, M., Petković, M., Ristivojević, P., Fira, D., & Berić, T. (2017). The profile and antimicrobial activity of Bacillus lipopeptide extracts of five potential biocontrol strains. Frontiers in Microbiology, 8, 925. https://doi.org/10.3389/fmicb.2017.00925

[8] Dobrzyński, J., Jakubowska, Z., Kulkova, I., Kowalczyk, P., & Kramkowski, K. (2023). Biocontrol of fungal phytopathogens by Bacillus pumilus. Frontiers in Microbiology, 14, 1194606. https://doi.org/10.3389/fmicb.2023.1194606

[9] Einloft, T. C., Oliveira, P. B., Radünz, L. L., & Dionello, R. G. (2021). Biocontrol capabilities of three Bacillus isolates towards aflatoxin B1 producer A. flavus in vitro and on maize grains. Food Control, 125, 107978. https://doi.org/10.1016/j.foodcont.2021.107978

[10] Gebreslassie, S., Jida, M., Puente, M. L., Covacevich, F., & Belay, Z. (2024). Inoculation of native arbuscular mycorrhizae and Bacillus subtilis can improve growth in vegetable crops. International Journal of Microbiology, 2024(1), 9226715. https://doi.org/10.1155/2024/9226715

[11] Gong, Q., Zhang, C., Lu, F., Zhao, H., Bie, X., & Lu, Z. (2014). Identification of bacillomycin D from Bacillus subtilis fmbJ and its inhibition effects against Aspergillus flavus. Food Control, 36, 8–14. https://doi.org/10.1016/j.foodcont.2013.07.034

[12] Grahovac, J., Pajčin, I., & Vlajkov, V. (2023). Bacillus VOCs in the context of biological control. Antibiotics, 12(3), 581. https://doi.org/10.3390/antibiotics12030581

[13] Guo, N., Wang, S., Whitfield, C. T., Batchelor, W. D., Wang, Y., Blersch, D., Higgins, B. T., Feng, Y., Liles, M. R., de-Bashan, L. E., Wang, Y., & Ma, Y. (2025). High-efficiency CRISPR-Cas9 genome editing unveils biofilm insights and enhances antimicrobial activity in Bacillus velezensis FZB42. Biotechnology and Bioengineering, 122(4), 983–994. https://doi.org/10.1002/bit.28933

[14] Iacovelli, R., Bovenberg, R. A. L., & Driessen, A. J. M. (2021). Nonribosomal peptide synthetases and their biotechnological potential in Penicillium rubens. Journal of Industrial Microbiology and Biotechnology, 48(7–8), kuab045. https://doi.org/10.1093/jimb/kuab045

[15] Iqbal, S., Begum, F., Rabaan, A. A., Aljeldah, M., Al Shammari, B. R., Alawfi, A., Alshengeti, A., Sulaiman, T., & Khan, A. (2023). Classification and multifaceted potential of secondary metabolites produced by Bacillus subtilis group: a comprehensive review. Molecules, 28(3), 927. https://doi.org/10.3390/molecules28030927

[16] Jan, F., Arshad, H., Ahad, M., Jamal, A., & Smith, D. L. (2023). In vitro assessment of Bacillus subtilis FJ3 affirms its biocontrol and plant growth promoting potential. Frontiers in Plant Science, 14, 1205894. https://doi.org/10.3389/fpls.2023.1205894

[17] Kaspar, F., Neubauer, P., & Gimpel, M. (2019). Bioactive secondary metabolites from Bacillus subtilis: a comprehensive review. Journal of Natural Products, 82, 2038–2053. https://doi.org/10.1021/acs.jnatprod.9b00110

[18] Kloepper, J. W., Ryu, C. M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology, 94(11), 1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

[19] Leclère, V., Béchet, M., Adam, A., Guez, J. S., Wathelet, B., Ongena, M., Thonart, P., Gancel, F., Chollet-Imbert, M., & Jacques, P. (2005). Mycosubtilin overproduction by Bacillus subtilis BBG100 enhances the organism's antagonistic and biocontrol activities. Applied and Environmental Microbiology, 71(8), 4577–4584. https://doi.org/10.1128/AEM.71.8.4577-4584.2005

[20] Li, S., Xu, X., Zhao, T., Ma, J., Zhao, L., Song, Q., & Sun, W. (2022). Screening of Bacillus velezensis E2 and the inhibitory effect of its antifungal substances on Aspergillus flavus. Foods, 11(2), 140. https://doi.org/10.3390/foods11020140

[21] Liu, Y., Teng, K., Wang, T., Dong, E., Zhang, M., Tao, Y., et al. (2020). Antimicrobial Bacillus velezensis HC6: production of three kinds of lipopeptides and biocontrol potential in maize. Journal of Applied Microbiology, 128, 242–254. https://doi.org/10.1111/jam.14459

[22] Maciag, T., Kozieł, E., Rusin, P., Otulak-Kozieł, K., Jafra, S., & Czajkowski, R. (2023). Microbial consortia for plant protection against diseases: more than the sum of its parts. International Journal of Molecular Sciences, 24, 12227. https://doi.org/10.3390/ijms241512227

[23] Mali, H., Shah, C., Raghunandan, B. H., Prajapati, A. S., Patel, D. H., Trivedi, U., & Subramanian, R. B. (2023). Organophosphate pesticides an emerging environmental contaminant: pollution, toxicity, bioremediation progress, and remaining challenges. Journal of Environmental Sciences, 127, 234–250. https://doi.org/10.1016/j.jes.2022.04.023

[24] Malik, J., Moosa, A., Zulfiqar, F., Aslam, M. N., Albalawi, M. A., Almowallad, S., Mahmood, T., Alasmari, A., & Yong, J. W. H. (2024). Biocontrol potential of lipopeptides produced by the novel Bacillus altitudinis strain TM22A against postharvest Alternaria rot of tomato. LWT – Food Science and Technology, 191, 115541. https://doi.org/10.1016/j.lwt.2023.115541

[25] Mardanova, A. M., Hadieva, G. F., Lutfullin, M. T., Khilyas, I. V., Minnullina, L. F., Gilyazeva, A. G., Bogomolnaya, L. M., & Sharipova, M. R. (2017). Bacillus subtilis strains with antifungal activity against the phytopathogenic fungi. Agricultural Sciences, 8, 1–20. https://doi.org/10.4236/as.2017.81001

[26] Markelova, N., & Chumak, A. (2025). Antimicrobial activity of Bacillus cyclic lipopeptides and their role in the host adaptive response to changes in environmental conditions. International Journal of Molecular Sciences, 26(1), 336. https://doi.org/10.3390/ijms26010336

[27] Martínez-Núñez, M. A., & López, V. E. L. (2016). Nonribosomal peptides synthetases and their applications in industry. Sustainable Chemical Processes, 4(1), 13. https://doi.org/10.1186/s40508-016-0057-6

[28] Masood, A. B., Badshah, M., Ali, M. I., Masood, K., Jamal, A., & Nicolaisen, M. (2025). Antifungal potential of non-ribosomal peptide producing Serratia surfactantfaciens (S31) and Alcaligenes pakistanensis (S33) isolated from the Solanum lycopersicum rhizosphere. Physiological and Molecular Plant Pathology, 139, 102778. https://doi.org/10.1016/j.pmpp.2025.102778

[29] Massucato, L. R., et al. (2022). Efficiency of combining strains Ag87 (Bacillus megaterium) and Ag94 (Lysinibacillus sp.) as phosphate solubilizers and growth promoters in maize. Microorganisms, 10(7), 1401. https://doi.org/10.3390/microorganisms10071401

[30] Meena, K. R., & Kanwar, S. S. (2015). Lipopeptides as the antifungal and antibacterial agents: applications in food safety and therapeutics. BioMed Research International, 2015, 473050. https://doi.org/10.1155/2015/473050

[31] Miljaković, D., Marinković, J., & Balešević-Tubić, S. (2020). The significance of Bacillus spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms, 8(7), 1037. https://doi.org/10.3390/microorganisms8071037

[32] Molina-Romero, D., Juárez-Sánchez, S., Venegas, B., Ortíz-González, C. S., Baez, A., Morales-García, Y. E., et al. (2021). A bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. Frontiers in Sustainable Food Systems, 4, 616757. https://doi.org/10.3389/fsufs.2020.616757

[33] Mora, I., Cabrefiga, J., & Montesinos, E. (2015). Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated Bacillus against phytopathogenic bacteria. PLoS ONE, 10(5), e0127738. https://doi.org/10.1371/journal.pone.0127738

[34] Mortinho, E. S., Jalal, A., da Silva Oliveira, C. E., Fernandes, G. C., Pereira, N. C. M., Rosa, P. A. L., do Nascimento, V., de Sá, M. E., & Teixeira Filho, M. C. M. (2022). Co-inoculations with plant growth-promoting bacteria in the common bean to increase efficiency of NPK fertilization. Agronomy, 12(6), 1325. https://doi.org/10.3390/agronomy12061325

[35] Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters, 170(1), 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

[36] Nkir, D., Aallam, Y., Ibnyasser, A., et al. (2024). Geographic bioprospection of maize rhizoplane-associated bacteria for consortia construction and impact on plant growth and nutrient uptake under low P availability. Environmental and Experimental Botany, 226, 105939. https://doi.org/10.1016/j.envexpbot.2024.105939

[37] Nysanth, N. S., Sivapriya, S. L., Natarajan, C., & Anith, K. N. (2022). Novel in vitro methods for simultaneous screening of two antagonistic bacteria against multiple fungal phytopathogens in a single agar plate. 3 Biotech, 12(6), 140. https://doi.org/10.1007/s13205-022-03205-3

[38] Premono, M. E., Moawad, A. M., & Vlek, P. L. G. (1996). Effect of phosphate-solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. Indonesian Journal of Crop Science, 11, 13–23.

[39] Puan, S. L., Erriah, P., Baharudin, M. M. A., Yahaya, N. M., Kamil, W. N. I. W. A., Ali, M. S. M., et al. (2023). Antimicrobial peptides from Bacillus spp. and strategies to enhance their yield. Applied Microbiology and Biotechnology, 107, 5569–5593. https://doi.org/10.1007/s00253-023-12651-9

[40] Qin, W. Q., Liu, Y. F., Zhou, L., Liu, J. F., Fei, D., Xiang, K. H., Yang, S. Z., Gu, J. D., & Mu, B. Z. (2025). Genome mining, structural elucidation and surface-active property of a new lipopeptide from Bacillus subtilis. Microbial Cell Factories, 24(1), 106. https://doi.org/10.1186/s12934-025-02723-y

[41] Saiyam, D., Dubey, A., Malla, M. A., & Kumar, A. (2024). Lipopeptides from Bacillus: unveiling biotechnological prospects—sources, properties, and diverse applications. Brazilian Journal of Microbiology, 55, 281–295. https://doi.org/10.1007/s42770-023-01228-3

[42] Salazar, B., Ortiz, A., Keswani, C., Minkina, T., Mandzhieva, S., Pratap Singh, S., et al. (2023). Bacillus spp. as bio-factories for antifungal secondary metabolites: innovation beyond whole organism formulations. Microbial Ecology, 86, 1–24. https://doi.org/10.1007/s00248-022-02044-2

[43] Sani, A., Qin, W. Q., Li, J. Y., Liu, Y. F., Zhou, L., Yang, S. Z., et al. (2024). Structural diversity and applications of lipopeptide biosurfactants as biocontrol agents against phytopathogens: a review. Microbiological Research, 278, 127518. https://doi.org/10.1016/j.micres.2023.127518

[44] Santoso, I., Fadhilah, Q. G., Maryanto, A. E., & Yasman, Y. (2021). Antagonist effect of Bacillus spp. against Aspergillus niger CP isolated from cocopeat powder. IOP Conference Series: Earth and Environmental Science, 846, 012001. https://doi.org/10.1088/1755-1315/846/1/012001

[45] Singh, D., Jadon, K. S., Verma, A., Geat, N., Sharma, R., Meena, K. K., & Kakani, R. K. (2024b). Formulations of synergistic microbial consortia for enhanced systemic resistance against Fusarium wilt in cumin. International Microbiology, 28(3), 497–523. https://doi.org/10.1007/s10123-024-00553-3

[46] Singh, S., Puri, S., & Sohal, S. K. (2024a). Alternatives to chemical pesticides: current trends and future implications. In Pesticides in the Environment (pp. 307–334). Elsevier. https://doi.org/10.1016/b978-0-323-99427-9.00003-3

[47] Tapi, A., Chollet-Imbert, M., Scherens, B., & Jacques, P. (2010). New approach for the detection of non-ribosomal peptide synthetase genes in Bacillus strains by polymerase chain reaction. Applied Microbiology and Biotechnology, 85(5), 1521–1531. https://doi.org/10.1007/s00253-009-2176-4

[48] Wang, L., Jia, S., Du, Y., Cao, H., Zhang, K., Xing, J., & Dong, J. (2025). Biocontrol potential of Bacillus subtilis A3 against corn stalk rot and its impact on root-associated microbial communities. Agronomy, 15(3), 706. https://doi.org/10.3390/agronomy15030706

[49] Wang, S., Xu, M., Han, Y., & Zhou, Z. (2024). Exploring mechanisms of antifungal lipopeptide iturin A from Bacillus against Aspergillus niger. Journal of Fungi, 10(3), 172. https://doi.org/10.3390/jof10030172

[50] Yuan, S., Wu, Y., Jin, J., Tong, S., Zhang, L., & Cai, Y. (2023). Biocontrol capabilities of Bacillus subtilis E11 against Aspergillus flavus in vitro and for dried red chili (Capsicum annuum L.). Toxins, 15(5), 308. https://doi.org/10.3390/toxins15050308

[51] Hussain, S., Qaisrani, S. A., Mubeen, M., Javeed, H. M. R., & Tahir, M. (2026). Synergistic Effects of Microbial Inoculation and Nitrogen Fertilization on Maize Performance and Yield Attributes. Nitrogen, 7(3), 87. https://doi.org/10.3390/nitrogen7030087

[52] Mubeen, M., Bano, A. Ali, B. Islam, Z.U. Ahmad, A. Hussain, S. Fahad S. and Nasim. W. (2021). Effect of plant growth promoting bacteria and drought on spring maize (Zea mays L.). Pak. J. Bot., 53(2): 731-739, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-2(38).

[53] Mubeen, M., Shabbir, K., Hanif, A., Ali, M., Hussain, S., & Ahmad, S. (2023). Role of Environmental Science for Disaster Risk Reduction in Agriculture. In Disaster Risk Reduction in Agriculture (pp. 131-145). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-1763-1_7.