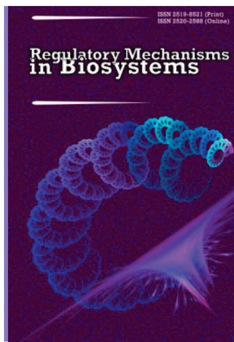

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Isolation of antagonistic bacterial strains against fungi

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This study focused on isolating and identifying antagonistic bacterial strains from diseased vegetable crops and evaluating their potential as biocontrol agents against phytopathogenic fungi. Samples were collected from tomato, sweet pepper, and potato plants across various regions of Uzbekistan. Several bacterial strains were identified using morphological, molecular, and biochemical analyses, with *Bacillus licheniformis* 6.25 emerging as the most potent antagonist. Molecular characterization through 16S rRNA sequencing confirmed a 99.86% similarity to known *B. licheniformis* species. The antagonistic activity of *B. licheniformis* 6.25 was tested against *Fusarium solani*, *Alternaria alternata*, and *Verticillium dahliae*. The strain suppressed fungal growth by over 50%, demonstrating strong antifungal properties. Metabolite analysis via GC-MS revealed bioactive compounds with antifungal, anti-inflammatory, and antimicrobial effects. Antifungal assays showed inhibition zones of 26 mm against *A. alternata* and 21 mm against *F. solani*. These findings highlight the potential of *B. licheniformis* 6.25 as a biocontrol agent for sustainable agriculture and pharmaceutical applications.

Keywords: *Bacillius*; bacteria; microflora; pathogens; antagonistic activity.

Introduction

It is known that the advancement of organic farming and the biological protection of plants against phytopathogenic diseases stand as pressing contemporary challenges. The isolation of natural antagonists from affected regions has emerged as a potent approach to biological protection. With the escalating environmental concerns surrounding chemical pesticides in the 21st century, there has been a notable surge in combating phytopathogenic plant diseases using environmentally friendly strains of natural antagonist microorganisms (Ongena & Jacques, 2008.). In addition to their biocontrol capabilities, various strains of microorganisms also exhibit plant growth-promoting properties. Notably, bacterial genera such as *Bacillus*, *Pseudomonas*, and *Agrobacterium* have gained prominence for their significant roles in enhancing soil health and promoting plant growth (Fira et al., 2018). Bacteria belonging to the *Bacillus* genus exhibit a widespread distribution, inhabiting diverse environments such as soil, water, and air. They are commonly found in various plant parts, including stems, leaves, and rhizospheres, as well as in the gastrointestinal tract of animals, among other ecological niches (Felske, 2004; Pignatelli et al., 2009; Connor et al., 2010). One of the important features of *Bacillus* species is their capacity to produce a wide array of secondary metabolites, including structurally diverse antagonistic substances, which have gained research attention. It is worth noting that around 4–5% of the genomes of *Bacillus subtilis* strains are responsible for the synthesis of secondary metabolites, enabling them to generate over 20 distinct chemical compounds with antimicrobial properties (Stein, 2005).

Bacillus licheniformis is a ubiquitous saprophytic bacterium found in nature, often employed in the fermentation industry for the synthesis of amylase, protease, antibiotics, and various valuable metabolites (US Environmental Protection Agency, 1997. *Bacillus licheniformis* final risk assessment, No. 3169). Bacteriocin is a peptide antibiotic originating from the *Bacillus licheniformis* bacterium, renowned for its antibacterial properties effective against a broad spectrum of bacterial species. It finds extensive applications in both medical and veterinary fields (Logan, 1988). The global promotion of biological agents in combatting plant diseases has recently gained significant traction. These biological preparations are known for their safety for human health, minimal environmental impact, and cost-effectiveness. The mechanism of action of these biopreparations against phytopathogenic microorganisms primarily involves antagonism, antibiotic production, and enzyme activity. This research aims to identify the most potent antagonistic bacterial strain for combating phytopathogenic microorganisms in vegetable crops and to investigate its antifungal capabilities.

Materials and methods

In this research, we assessed how well certain bacteria, which were obtained from infected vegetable crops across various regions in Uzbekistan, can combat the fungi that were isolated from infected vegetable crops in the same regions. These fungi were chosen as the target strains for our study to evaluate the effectiveness of the bacteria in fighting them (Table 1).

Table 1
The origin of the fungi and bacteria used in the study

Species, strain	Separated substrate	Place of (district, region)
Stenotrophomonas sp 2/10	tomato leaf	Kibrai, Tashkent
Bacillus licheniformis 6/25	sweet pepper leaf	Shakhrisabz, Kashkadarya
Bacillus pumilus 2/17	tomato stalk	Kibrai, Tashkent district
Bacteroides ovatus 4/22	potato leaf	Kasbi, Kashkadarya
Pseudomonas putida 4/23	potato leaf	Kasbi, Kashkadarya
Pseudomonas alcaliphila 2/18	tomato stalk	Kibrai, Tashkent
Phytium sp. 1/7	sweet pepper stalks	Kibrai, Tashkent
Fusarium oxysporium 3/1	tomato root	Kuva, Fergana
Fusarium solani 1/5	sweet pepper root	Kibrai, Tashkent
Penicillium sp. 3/4	tomato rhizosphere	Kuva, Fergana
Alternaria.alternata 4/11	potato leaf	Kuva, Fergana
Alternaria sp 2/21	tomato fruit	Kibrai, Tashkent
Fusarium culmorum 1/6	sweet pepper root	Kibrai, Tashkent
Verticilium dahliae 1/22	tomato leaf	Kibrai, Tashkent

Isolation of microorganisms from diseased plant samples. First, we collected samples of infected vegetable crops and brought them to the laboratory. Next, we carefully cut the infected leaves, stems, and root parts of the plants. These plant parts were then washed in running water and soaked in a 3% hydrogen peroxide solution for 2–3 minutes. Afterward, they were thoroughly rinsed with sterile tap water. Once they were dry, we took 0.5–1.0 cm sections of leaves, stems, and roots that contained both visibly affected and healthy tissues. These plant sections were placed in Petri dishes filled with Chapek and MPA media for further analysis.

Similarly, after externally sterilizing the plant parts, we crushed them separately using sterile porcelain mortars. These crushed samples were then placed in Petri dishes containing Chapek and GPA medium. The samples placed in the Chapek medium were kept in an incubator at a temperature of 24–25 °C, while those in the MPA nutrient medium were placed in a thermostat at a temperature of 27–28 °C. We monitored the growth of fungi for a period of 2–7 days and observed the growth of bacteria for 12–72 hours. Through repeated cultivation, we were able to isolate pure cultures of these microorganisms for further analysis.

Identifying the fungi. We conducted a morphological examination using an NLCD-307B-2 microscope (Hinotek, China). The examination was performed at a magnification of 400 times, and we employed conventional methods as described in the relevant literature to identify the fungal species (Sagdullaeva et al., 1999; Bilai et al., 2006; Sokirko et al., 2014).

Dual culture analysis. To assess their antagonistic potential, we conducted a study in which strains of phytopathogenic fungi were evaluated in their interaction with *B. licheniformis* 6.25. For this analysis, we extracted cylindrical blocks with a 6 mm diameter from both the antagonistic and phytopathogenic strains. These blocks were carefully positioned on opposite sides of a Petri dish, with a 5.5 mm gap between them. We repeated this experimental arrangement three times to ensure precision. Subsequently, the Petri dishes were placed in a thermostat set at a consistent temperature of 25 °C. After 7 days, we quantified the radius of the antagonistic zone that had developed between the two strains. The radius calculation was executed using a dedicated formula to obtain accurate results (Mao et al., 2020):

$$\text{Growth inhibition rate (\%)} = \frac{\text{Control colony radius} - \text{Treatment colony radius}}{\text{Control colony radius}} \times 100$$

Identification of bacteria. In traditional bacterial identification, classic methods were employed. This included close observation of the structure, color, and size of individual bacterial colonies that had been isolated in pure form. Additionally, fixed slides of these isolates were prepared and subjected to the Gram's staining method. These stained slides were then examined using a microscope, specifically the XSP-136 B and N-300M (UCMOSO9000KPB) brand microscope, which provided a magnification of 1000 times, utilizing immersion oil for enhanced clarity (Hucker et al., 1927). Furthermore, the identification of bacteria was conducted using two methods: MALDI-TOF with Bruker equipment (Lowe et al., 2018; Stepanov et al., 2018) and 16S rRNA gene phylogeny analysis. The systematic classification of the isolated bacteria was determined following Bergey's taxonomy (Hoult et al., 1997).

The antagonistic strain. Stenotrophomonas sp. 2/10, *Bacillus licheniformis* 6/25, *Bacillus pumilus* 2/17, *Bacteroides ovatus* 4/22, *Pseudomonas putida* 4/23, *Pseudomonas alcaliphila* 2/18 were cultivated in MPA medium within Petri dishes at a temperature of 28 °C for 4 days.

Indicator strains. In this study, indicator strains were employed for the antagonism research. These strains, previously isolated from infected parts of potato, tomato, and sweet pepper crops (Khaitbaeva et al., 2023), include *Phytium* sp. 1/7, *Penicillium* sp. 3/4, *Fusarium oxysporium* 3/1, *Alternaria* sp. 2/21, *F. culmorum* 1/6, *Verticillium dahliae* 1/22, *Alternaria alternata* 4/11, and *Fusarium solani* 1/5. The phytopathogenic fungi were cultured on Czapek Dox Agar medium, which had the following composition per liter: sucrose – 30.0 g, sodium nitrate – 3.0 g, dipotassium phosphate – 1.0 g, magnesium sulfate – 0.5 g, potassium chloride – 0.5 g, ferrous sulfate – 0.01 g, with a pH adjusted to 7.3. The cultivation of these fungi took place at a temperature of 25 °C, and they were allowed to grow for 8 days.

Assay of antagonistic activity. The antagonistic activity was assessed using a method involving the diffusion of substances into agar wells under aerobic conditions (He et al., 2006). In this experiment, an inoculum of 10^7 colony-forming units (CFU) of the relevant indicator microorganism was applied to pre-prepared agar plates and allowed to air dry. Subsequently, wells with a 6 mm diameter were created in the agar plates using a cork drill. These wells were then filled with 20 µL of the *B. licheniformis* 6.25 extract. Afterward, the plates were incubated for a period ranging from 18 to 72 hours, depending on the specific type of microorganism, at a temperature of 25 °C. The antagonistic zones that formed around the wells were measured using an antibiotic zone scale (Himedia).

DNA isolation and PCR amplification of the 16S rRNA gene. Genomic DNA from *B. licheniformis* 6.25 was isolated using the modified Marmur (Salvà-Serra et al., 2018) method (Fig. 1). PCR amplification was performed using universal oligonucleotide primers of the 16S rRNA gene: 27F-AGAGTTTGATCMTGGCTCAG, and 1492R-GGTTACCTTGTTACGACTT (Lane, 1991).

PCR amplification of the DNA samples isolated from the bacterial strain was carried out in the GenPak® PCR MasterMix kit. In this case, the reaction was prepared in a total volume of 20 µL, consisting of 10 µL of Dilution, 8.2 µL of double-distilled water, 0.4 µL of primer (27F and 1492R), and 1 µL of DNA. PCR amplification optimization initial denaturation at 94 °C for 3 minutes, denaturation at 94 °C for 40 seconds, primer annealing) at 55 °C for 40 seconds, elongation at 70 °C for 90 seconds in repeated 35 cycles, and final elongation at 70 °C for 7 minutes was conducted. The amplicons were detected by electrophoresis on a 1% agarose gel stained with ethidium bromide (Fig. 2).

PCR product purification and sequencing. For sequencing, PCR products were excised from 1% agarose gel and purified using the QIAquick® Gel Extraction Kit manual [HB-09010031114358_PCard_QQ_PCR_Gel_Cleanup _Kit_0718_WW%20]. The quantity of purified PCR products was measured in a NanoDrop device (NanoPhotometer® N60; IMPLEN). Sequencing of the samples was performed using BigDye Terminator v.3.1 cycle sequencing kit and Applied Biosystems® Genetic Analyzers, 3130 series sequence (Thermo Fischer Scientific, USA). The sequence result of this *B. licheniformis* 6.25 was cross-compared with the species in the NCBI BLAST database (www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree of *B. licheniformis* 6.25 based on 16S rRNA gene region was built via MEGA-X software (version 10.1.8) (Tamura et al., 2007).

Filtration. After four days of growth, the *B. licheniformis* 6.25 strain was isolated from the culture. This was achieved through a double filtration process employing a 0.22 µm membrane filter. The culture fluid, separated from the biomass, was subsequently stored at 4 °C for future applications.

Extraction. The separated culture liquid underwent a double extraction process using a separating funnel, with a ratio of 3 parts ethyl acetate (EtOAc) to 1 part culture liquid. This extraction cycle was repeated every 2 hours. After each extraction, the aqueous layer that settled at the bottom of the separating funnel was meticulously removed. Following this, the ethyl acetate extract was subjected to a drying process. This entailed heating it at 40 °C under vacuum conditions, typically carried out with a rotary evaporator. This drying phase effectively eliminated any remaining solvents, leaving behind the desired compounds or substances from the culture liquid (Stracquadanio et al., 2020).

Analysis of VOCs. To identify unknown volatiles, we employed a YL 6900 GC/MS (Gas Chromatograph/Mass Spectrometer) system from Young In Chromass in Korea. We used a DB-5MS column with dimensi-

ons of 30 meters in length, 0.25 mm internal diameter, and a film thickness of 0.25 µm. Oven temperature: Initially set at 80 °C for 3 minutes, then increased at a rate of 15 °C per minute up to 250 °C, with a 3-minute hold. Carrier gas: Helium was used at a flow rate of 1.0 mL/min. Evaporator temperature: 280 °C. Flow section: 1/20. Analysis time: 17 minutes. Injection method: Liquid samples were injected using a 1 µL microsyringe. Transmission line temperature: 300 °C. Ionization voltage: –70 eV. Ion source temperature: 230 °C. Scanning range: 30–350 a.m.u. To identify the components, we compared the mass spectra of each component obtained during the analysis with available spectral data from the NIST 2017 MS library. This allowed us to determine the identity of the unknown volatiles present in the samples (Guarrasi et al., 2017).

Results

Microorganisms serve as valuable natural biocontrol agents in diverse fields, encompassing laboratory research, agriculture, medicine, and forestry. Within this array, the genus *Bacillus* is extensively harnessed for shielding plants against both phytopathogenic microorganisms and insects. In the greenhouses situated in the Shakhrisabz district, nestled within the Kashkadarya region, "Vidrona" sweet pepper plants encountered notable infection rates resulting from phytopathogenic diseases. Specifically, *Fusarium* constituted 20.5%, *Phytophthora* 30.7%, *Alternaria* 10.2%, and bacterial rot 5.52% of the observed infections. These infections manifested in various plant parts, spanning leaves, stems, and roots, underscoring the pervasiveness of phytopathogenic microorganisms in this context (Fig. 1).

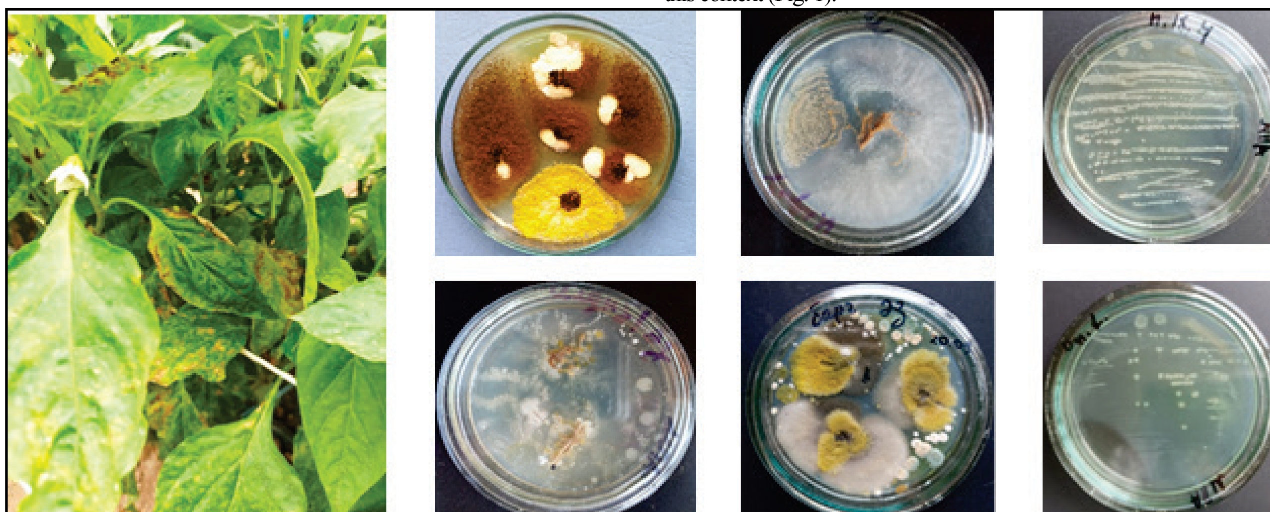

Fig. 1. Isolated microorganisms from "Vidrona" sweet pepper plants

Beyond the isolation of phytopathogens, our study yielded a promising outcome with the isolation of antagonistic microorganisms. These microorganisms, intriguingly, demonstrated a dual nature, being both pathogenic and antagonistic. Systematically, we classified them into two distinct groups. Employing sophisticated microbiological techniques, we successfully isolated pure bacterial cultures from the leaves of sweet pepper plants. Through a meticulous screening process, a particular bacterial strain, identified as *Bacillus* sp. 6.25, stood out due to its remarkable antagonistic properties. This finding holds great promise in combating phytopathogenic microorganisms. The selection of *Bacillus* sp. 6.25 for further investigation was primarily driven by its outstanding antagonistic potential. To assess its effectiveness in combatting phytopathogenic strains, micromycetes isolated from the same "Vidrona" sweet pepper plants, which were unequivocally identified as phytopathogenic, were employed as indicators. Our research endeavors revealed that *Bacillus* sp. 6.25 demonstrated substantial antagonistic activity against these phytopathogenic fungi. This significant finding highlights the promising role of *Bacillus* sp. 6.25 as a potential biocontrol agent for managing phytopathogenic infections in sweet pepper plants.

Microorganisms, encompassing both bacteria and fungi, were extracted from various parts of infected vegetable crops, including leaves, stems, and roots, sourced from diverse regions within our republic. A comprehensive analysis of these isolated microorganisms, in conjunction with pertinent data from existing literature sources, revealed the coexistence of antagonistic microorganisms alongside phytopathogens. Additionally, we ascertained the pathogenic nature of the isolated fungi by assessing their phytotoxicity traits (Azimova et al., 2023; Khaitbaeva et al., 2023), and these isolated bacteria were subsequently employed as phytopathogenic fungi in our experimental setup. The central aim of our study was to investigate the antagonistic properties of these isolated bacteria (Table 2).

Our observations revealed a varied spectrum of interactions between the isolated bacteria and phytopathogenic fungi. Most bacteria demonstrated antagonistic relationships with the fungi, signifying their capability to inhibit fungal growth. However, there were instances of symbiotic interactions between specific bacteria and fungi. Notably, *Pseudomonas alcaliphila* exhibited antagonism against seven different fungi, with a significant impact on strain 2/18. In contrast, *Bacillus* sp. 6.25 emerged as a potent antagonist, effectively inhibiting the growth of all the studied phytopathogenic fungi. This strain excelled by creating antagonistic zones that achieved over 50% inhibition against fungi like *Phytium* sp. 1/7 and *Fusarium oxysporum* 3/1. It also reached a 50% inhibition rate against *Alternaria* sp. 2/21, *Verticillium dahliae* 1/22, *Penicillium* sp. 3/4, *Fusarium culmorum* 1/6, *Alternaria alternata* 4/11, and *Fusarium solani* 1/5 phytopathogenic strains. These findings underscore the remarkable potential of *Bacillus* sp. 6.25 as a biocontrol agent against a broad spectrum of plant pathogens (Fig. 2). As a result of this research, it is evident that *Bacillus* sp. 6.25 displayed the most remarkable antagonistic activity among the tested bacterial strains.

Throughout our research, it became evident that *Bacillus* sp. strain 6.25 exhibited substantial antagonistic activity against phytopathogenic fungi. Further examinations focused on characterizing the strain's morphocultural features. Our approach involved employing the Gram staining method, microscopic assessment using immersion oil, morphological analysis (Fig. 3), and taxonomic identification through the MALDI-TOF method. The selected strain, chosen based on its morphological characteristics, was identified as gram-positive and demonstrated mobility. It exhibited cell morphology with rounded tips, with some cells displaying slight curvature, measuring approximately 0.25–0.40 µm in length and 0.10–0.12 µm in width. A distinctive feature was the formation of spores originating from the center and transitioning from an elliptical to a cylindrical shape. When cultivated on MPA medium, this strain developed into small, wrinkled, opaque colonies with a sticky texture (Fig. 3).

The identification of the selected bacterial strain involved a multifaceted approach. It commenced with the examination of morphological characteristics, followed by a more in-depth analysis using the MALDI-TOF method. Through the integration of these complementary methods, the chosen strain was definitively identified as *Bacillus licheniformis*.

Table 2
The extent of inhibition (%) in the growth of phytopathogenic fungi by bacteria

Antagonists	Phytopathogens							
	Phytium sp. 1/7	*F. oxysporium*3/1	*F. solani* 1/5	*V. dahliae* 1/22	*Penicillium* sp. 3/4	*A. alternata*4/11	*Alternaria* sp. 2/21	*F. culmorum* 1/6
Stenotrophomonas sp. 2/10	0	13	11	0	0	17	0	0
Bacillus licheniformis 6.25	54	55	44	50	41	46	50	43
*Bacillus pumilus*2/17	11	0	0	12	0	0	0	0
Bacteroides ovatus 4/22	51	0	8	11	0	7	6	0
Pseudomonas putida 4/23	16	0	0	0	0	13	33	0
Pseudomonas alcaliphila 2/18	28	12	19	26	10	7	28	0

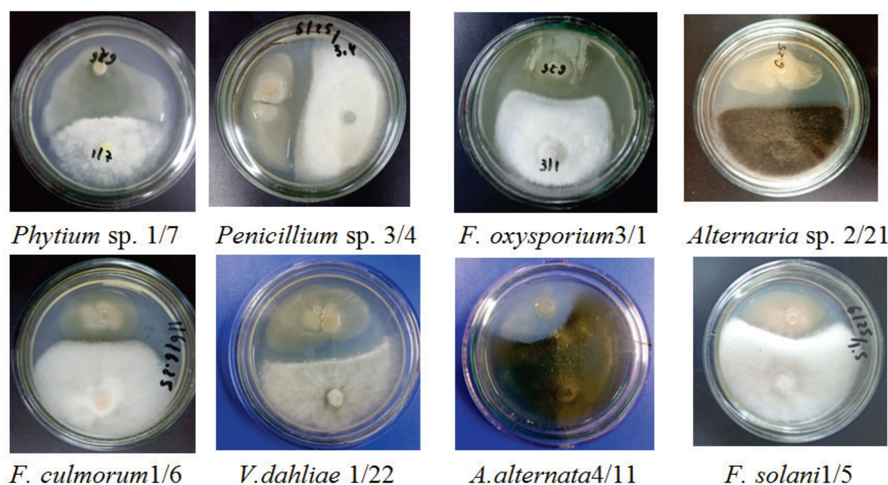

Fig. 2. Antagonistic interaction of *Bacillus* spp. 6.25 bacterium with phytopathogenic micromycetes *B. licheniformis* 6.25 identification

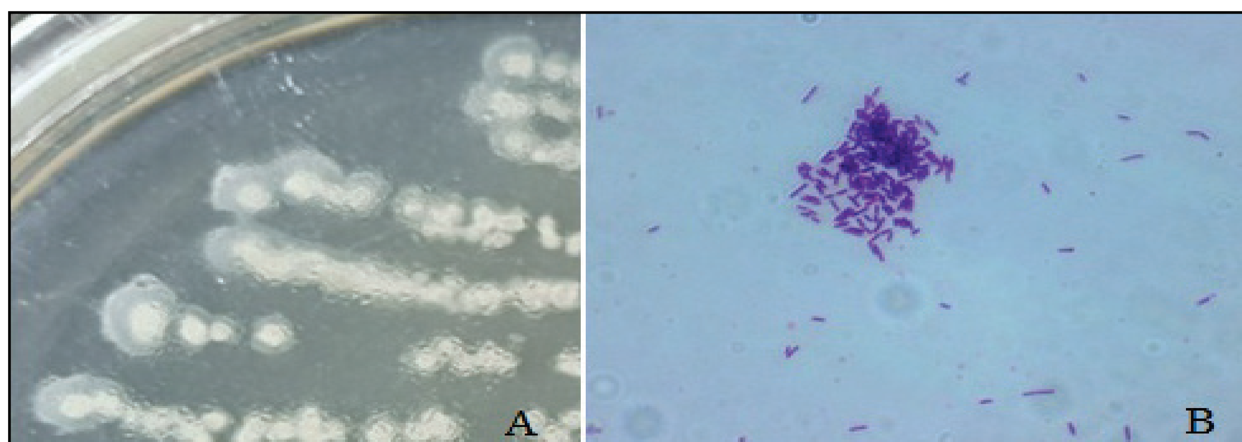

Fig. 3. Colony (*a*) and microscopic view (*b*) of *Bacillus* spp. 6.25

In contemporary microbiology, exclusive reliance on morphological characteristics for species identification may introduce ambiguity into the results. To bolster the scientific robustness of our research, we incorporated a molecular genetic method for identifying *B. licheniformis* 6.25. The procedure involved the extraction of DNA from the chosen strain, with a specific focus on the 16S rDNA gene, which served as a co-amplifier. PCR (Polymerase Chain Reaction) was conducted using the bacterium's genomic DNA. Subsequently, the PCR product underwent assessment and confirmation through agarose gel electrophoresis, a widely employed technique for validating the presence and size of DNA fragments in a given sample. This molecular genetic approach furnished a more precise and dependable means of strain identification (Fig. 4).

Figure 4a demonstrates the successful isolation of high-quality and pure genomic DNA from the *B. licheniformis* 6.25 bacterial strain, using a modified version of the Marmur method. In Figure 4b, it is shown that a 16S rRNA gene fragment of approximately 1500 base pairs (bp) was generated for *B. licheniformis* 6.25. Subsequently, the sequencing product of the 16S rRNA gene was determined to be 1422 bp in length. Comparative analysis with the NCBI BLAST database revealed a remarkable 99.9% similarity between this strain and approximately 100 strains of *B. licheniformis*. Consequently, we have designated this strain as *B. licheniformis* 6.25 (OR349680) based on the molecular identification of the 16S rRNA gene.

A phylogenetic tree was diligently assembled utilizing the Maximum-Likelihood statistical method, facilitated by the Mega4 bioinformatics software (Fig. 6). During the construction of this phylogenetic tree, *B. licheniformis* 6.25 underwent comprehensive analysis alongside other prominent *Bacillus* species, which encompassed *B. licheniformis*, *B. mojavensis*, *B. malacitensis*, *B. vallismortis*, *B. pumilus*, *B. subtilis*, and an outgroup represented by *E. coli*. This rigorous analysis provided invaluable insights into the evolutionary context of *B. licheniformis* 6.25 within the broader spectrum of related bacterial species (Fig. 5).

The phylogenetic tree analysis confirmed that the isolated strain belongs to the *Bacillus* genus, with branches of the tree showing a 100% similarity to other *B. licheniformis* strains. This aligns with the identification results obtained through morphological characteristics, the MALDI-TOF method, and molecular genetic methods, all of which consistently identified the strain as *B. licheniformis*.

In subsequent studies, the culture fluid of *B. licheniformis* 6.25 was extracted using ethyl acetate. This extract was then subjected to Gas Chromatography-Mass Spectrometry GC-MS analysis (Fig. 6).

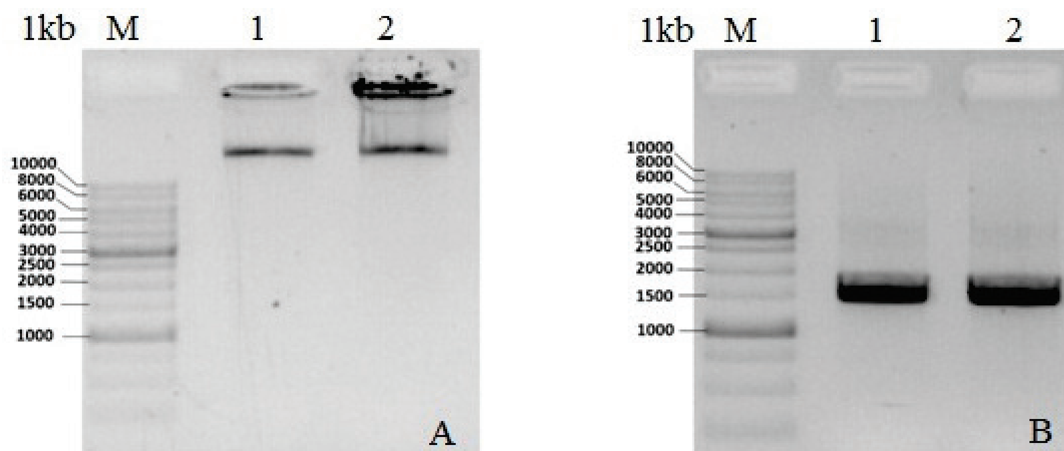

Fig. 4. Agarose gel electrophoresis: *a* – lane M: 1-kilobase DNA marker; Lanes 1 and 2: Genomic DNA of *B. licheniformis* 6.25; *b* – lane M: 1-kilobase DNA marker; Lanes 1 and 2: 16S rDNA PCR product of *B. licheniformis* 6.25

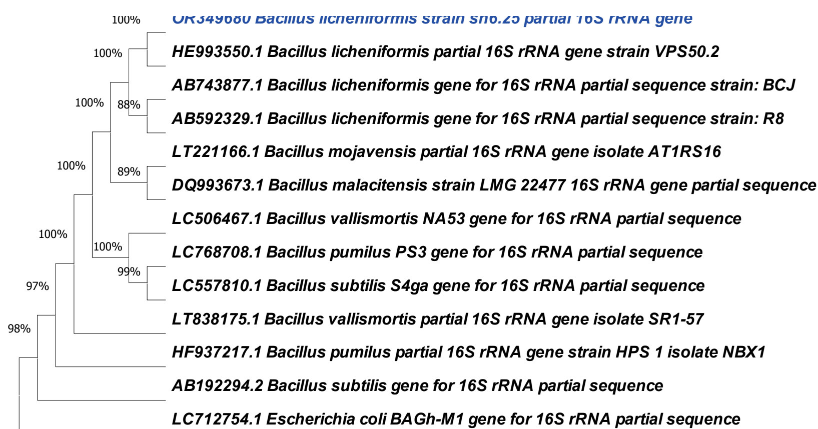

Fig. 5. The phylogenetic tree of *B. licheniformis* 6.25 was constructed using the Maximum-likelihood statistical method within the Mega4 bioinformatics software

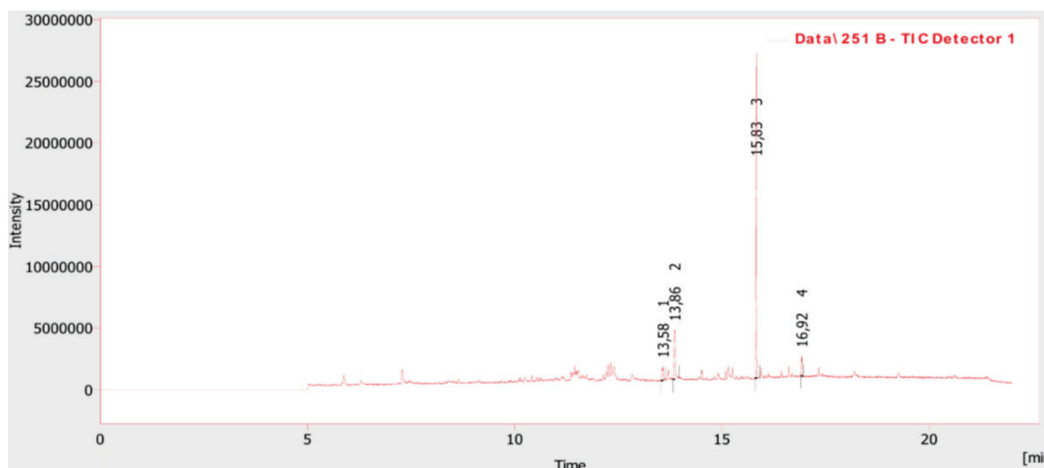

Fig. 6. Gas chromatography of *B. licheniformis* 6 25 culture fluid extract

In the conducted GC-MS analysis, the extract contains 1-heptatriacotanol (13.58), 7-Methyl-Z-tetradecen-1-ol acetate (13.86), bis(2-ethylhexyl) phthalate (15.83), 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl)ester (16.92) was found to have metabolites with antibacterial and antifungal properties. Antifungal activities of the isolated extract were conducted against phytopathogenic fungi *F. solani* and *A. alternata* (Table 3).

Table 3
Antifungal activity of *B. licheniformis* 6.25 culture fluid extract

Phytopathogenic fungi	1000 mg/mL	500 mg/mL	100 mg/mL
F. solani	21 ± 0.5	14 ± 0.2	6 ± 0.9
A. alternata	26 ± 0.7	7 ± 0.3	0 ± 0.0

The comprehensive extract exhibited a substantial 21 mm zone of inhibition at 1000 µg/mL when tested against *F. solani*. Notably, a noteworthy antagonistic effect was observed at 1000 µg/mL, resulting in a substantial 26 mm zone of inhibition against *A. alternata* fungus (Fig. 7). This outcome underscores the potent antifungal activity of the extract, particularly at higher concentrations.

The metabolites derived from the extraction process demonstrated remarkable antifungal activity at a concentration of 1000 µg/mL. This potent antifungal activity was directed against phytopathogens responsible for plant diseases. These findings hold promise for the application of metabolites produced by *B. licheniformis* 6.25 in the realm of biocontrol agents

and the development of strategies for managing plant diseases. Their ability to effectively inhibit the growth of these detrimental fungi positions them as valuable assets in safeguarding plant health and crop productivity.

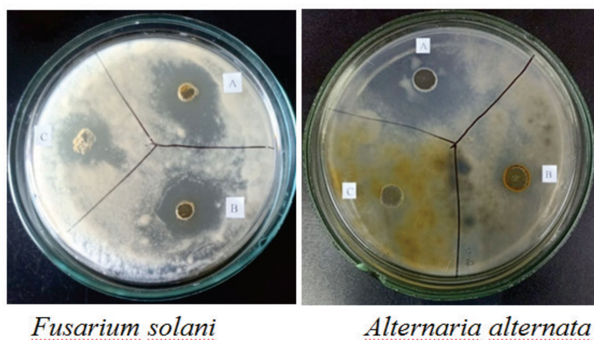

Fig. 7. Antifungal activity of *B. licheniformis* 6.25 culture fluid extract: *a* – 1000 µg/mL, *b* – 500 µg/mL, *c* – 100 mg/mL

Discussion

Microorganisms, particularly those of the genus *Bacillus*, are valuable natural biocontrol agents utilized across various domains, including agriculture, medicine, and forestry. They play a crucial role in safeguarding plants against the harmful impacts of phytopathogenic microorganisms and insects.

Vegetable products hold immense significance in human diets, serving as essential components of our food intake. Ensuring a robust and high-quality vegetable harvest is contingent on effective protection against various diseases that can afflict these crops. Our studies focused on investigating phytopathogenic diseases affecting vegetable crops, with a particular emphasis on sweet peppers. The research unveiled that sweet pepper plants are notably susceptible to diseases caused by representatives of the *Alternaria*, *Verticillium*, *Penicillium*, and *Fusarium* genera. Alternariosis, verticilliosis, and *Fusarium* infections, as documented in existing literature, are recurrent challenges faced by sweet pepper cultivation. Multiple scholarly works have underscored that these plant diseases, including those impacting sweet peppers, are primarily transmitted through the soil, significantly influencing crop productivity (Tsuchiya, 1990; Lorenzini et al., 1997; Nogués et al., 2002; Turaeva et al., 2021).

Bacillus licheniformis 6.25, a strain exhibiting significant antagonistic capabilities, was isolated from an infected sweet pepper plant in the company of disease-causing microorganisms. However, it is important to note that identifying species within the *Bacillus* genus based solely on morphological characteristics can be challenging. Many *Bacillus* species display considerable phenotypic variation, making species-level identification through morphology alone insufficient. In contemporary microbiology, the analysis of the 16S RNA gene, which is universally present in prokaryotes, has emerged as the standard method for studying bacterial phylogeny and taxonomy, offering a more precise and reliable means of species identification (Vardhan et al., 2011).

Through the analysis of the nucleotide sequence of the 16S rRNA gene and a cross-reference with the international GenBank database, our investigation confidently categorizes the studied strain within the *Bacillus* genus, demonstrating a high confidence level of 99.86%. Furthermore, this nucleotide sequence analysis led to the precise placement of the strain within the species *B. licheniformis*, providing a robust taxonomic identification.

In the intricate interplay of plant-microorganism interactions, it is noteworthy that approximately 80% of plants on our planet engage in symbiotic relationships with fungi, as established by the research of Karandashov et al. (2004). However, it is a delicate balance, as these fungi can sometimes transition into plant pathogens, jeopardizing the harmonious cooperation. The impact of fungal diseases on plants is often severe, surpassing the damage caused by other phytopathogenic microorganisms (Hussain & Usman, 2019). In this complex ecological web, it is fascinating to discover that antagonistic species against these phytopathogenic microorganisms coexist with the pathogens in natural settings. The isolation of these antagonistic microorganisms from naturally infected plant sources holds promise for developing innovative strategies in plant disease management and crop protection.

Over the past three decades, the extensive use of fungicides to combat phytopathogenic diseases has disrupted the delicate balance of microorganisms within the soil and plant ecosystems. This has, in turn, led to the emergence of fungicide-resistant microorganisms. In this study, *B. licheniformis* 6.25, isolated from infected sweet pepper leaves, emerges as a valuable player in providing microbiological protection to its host plant while fostering a symbiotic relationship. Notably, the selected *B. licheniformis* 6.25 demonstrated remarkable antifungal activity, inhibiting the growth of the micromycetes employed as indicator strains by more than 40%. Members of the *Bacillus* genus contribute to plant protection by producing a range of cyclic lipopeptides (Zeriouh et al., 2011).

These cyclic lipopeptides, classified as iturin and fengycin, are known for their potent antifungal activity (Romero et al., 2007). Their efficacy is associated with the composition of membranes, particularly the content of phospholipids and sterols (Falardeau et al., 2013). Lipopeptides produced by *Bacillus* species have demonstrated antagonistic effects against various fungi, including *Botrytis cinerea* (Zhang et al., 2013), *Candida albicans* (Song et al., 2013), and *Fusarium graminearum* (Zhao et al., 2014). These findings underscore the potential of *B. licheniformis* 6.25 as a valuable biocontrol agent against phytopathogenic fungi.

The results, in conjunction with existing literature, strongly indicate that *B. licheniformis* 6.25 possesses remarkable bactericidal and fungicidal properties, positioning it as a promising candidate for the development of antimicrobial biopreparations. Metabolite analysis of this bacterium revealed the presence of notable compounds, including 1-heptatriacotanol, a natural triterpene with demonstrated bioactivity in fields such as cytotoxicity, cardiovascular diseases, inflammation, and antiviral activity (Silva et al., 2012; Shavkiev et al., 2022). Additionally, 7-methyl-Z-tetradecen-1-ol acetate has been associated with anti-inflammatory, anti-cancer, and hepatoprotective effects (Imad et al., 2015), while 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester is known for its antimicrobial, anti-inflammatory, and antihyperlipidemic properties (Jasmine et al., 2013; Saravanakumar et al., 2016; Eshboev et al., 2023). These findings highlight the pharmaceutical and biotechnological potential of *B. licheniformis* 6.25 and its metabolites for diverse applications.

Metabolites extracted from *B. licheniformis* 6.25 underwent rigorous analysis via GC-MS, confirming the presence of biologically active compounds. Subsequent evaluation of the total extract's antifungal properties demonstrated its remarkable efficacy against prominent phytopathogens like *F. solani* and *A. alternata*. These findings underscore the potential of *B. licheniformis* 6.25-derived metabolites for applications in biocontrol and pharmaceuticals.

Conclusion

The study's findings reveal that infected vegetable crops contain both phytopathogenic microorganisms and valuable antagonistic microorganisms. Among the isolated bacteria, *B. licheniformis* 6.25 demonstrated remarkable antagonistic properties against phytopathogenic microscopic fungi. Molecular genetic techniques confirmed the identity of this strain as *B. licheniformis* with an impressive 99.9% similarity.

Moreover, *B. licheniformis* 6.25's culture fluid extract exhibited potent bioactivity against phytopathogenic micromycetes such as *F. solani* and *A. alternata*. The volatile metabolites within the extract displayed significant biological and pharmacological activities, targeting inflammation, microbes, and viruses. GC-MS analysis identified specific compounds such as 1-heptatriacotanol, 7-methyltetradecane-1-ol acetate, and 1,3-benzenedicarboxylic acid, bis(2 ethylhexyl) ester. Consequently, these findings highlight the potential of *B. licheniformis* 6.25 as a bio-fungicide for agriculture and suggest that its metabolites could serve as valuable antimicrobial compounds for pharmaceutical applications.

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