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BIOCHEMICAL AND ANTAGONISTIC PROPERTIES OF BACTERIA ISOLATED FROM A HOT SPRING AND THE COTTON RHIZOSPHERE

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ABSTRACT

This study investigated the biochemical and antagonistic properties of thermophilic and thermotolerant bacteria isolated from the Rayim So'fi hot spring in Kashkadarya Region, Uzbekistan, and cotton rhizosphere soil. Based on their phenotypic characteristics, two isolates were provisionally identified as *Geobacillus* sp. and *Bacillus* sp. Both isolates exhibited proteolytic, amylolytic, and catalase activities, whereas no lipolytic activity was detected. In vitro assays demonstrated that the bacterial isolates inhibited the mycelial growth of the phytopathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani*. These findings indicate that the investigated bacterial isolates may represent promising candidates for the development of biological plant protection strategies.

Keywords: Thermophilic Bacteria, Thermotolerant Bacteria, *Geobacillus* Sp., *Bacillus* Sp., Phytopathogenic Fungi, Antagonistic Activity, Biological Control.

1 INTRODUCTION

Plant diseases caused by fungi, bacteria, and viruses are among the major factors limiting agricultural crop productivity and are estimated to account for approximately 16% of global crop losses [1]. In particular, soil-borne diseases, including seedling, root, and root collar rots, as well as vascular wilting, can result in yield losses exceeding 50% under certain environmental conditions [2]. Chemical fungicides, such as metalaxyl, captan, benomyl, chlorothalonil, and copper oxychloride, are widely used worldwide to control diseases caused by phytopathogens belonging to genera such as *Pythium*, *Fusarium*, *Phytophthora*, *Rhizoctonia*, and *Sclerotium* [3,4].

However, the excessive and repeated application of chemical fungicides may lead to various environmental problems. These include contamination of soil and groundwater, suppression of beneficial soil microbial communities, adverse effects on mycorrhizal associations, and disruption of nutrient cycling processes in soil ecosystems. In particular, disturbances in microbiological processes associated with the biogeochemical nitrogen cycle have been reported as one of the potential adverse effects of chemical fungicides on soil ecosystems [3–5].

In this context, the development and improvement of biological control strategies against soil-borne phytopathogens represent a promising approach to sustainable plant protection. In particular, the application of bacteria with antagonistic properties offers considerable potential for suppressing the development of fungal diseases in agricultural crops. For example, the biocontrol efficacy of *Bacillus* subtilis strain GB-03 against *Fusarium oxysporum* f. sp. vasinfectum and *Rhizoctonia solani* in cotton has been investigated [6]. Similarly, the potential of *Bacillus* cereus for the biological control of *Phytophthora* sojae in soybean has been reported [7]. Furthermore, the reduced viability and biological activity of certain antagonistic bacteria under unfavorable environmental conditions, together with their limited capacity to provide long-term control of phytopathogens, represent major challenges to the practical application of biological control agents [8]. Therefore, the exploration of bacteria capable of tolerating elevated temperatures and other abiotic stress factors while exhibiting antagonistic activity against phytopathogenic fungi, as well as the investigation of their biological properties, is of considerable scientific importance.

Thermophilic bacteria are characterized by their ability to grow and maintain metabolic activity under high-temperature conditions. Some representatives of this group are capable of producing endospores, a characteristic that contributes to their survival under unfavorable environmental conditions. These physiological properties provide a basis for investigating thermophilic bacteria as potential biological control agents against plant diseases [3,4]. Furthermore, the ability of certain *Bacillus* species to colonize seed and root surfaces and suppress the development of phytopathogenic fungi highlights their importance in biological plant protection [9].

Thermophilic bacteria have been reported to occur in various natural and anthropogenic environments, including milk and dairy products [9,10], livestock waste compost [11], and the rhizosphere soils of agricultural crops [12]. However, the antagonistic properties of thermophilic and thermotolerant bacteria isolated from different ecological sources against soil-borne phytopathogenic fungi, as well as their potential applications in biological control, require further investigation.

The aim of this study was to isolate thermophilic and thermotolerant bacteria from a hot spring and cotton (*Gossypium hirsutum* L.) rhizosphere soil, characterize their morphological, physiological, and biochemical properties, and evaluate their antagonistic activity against selected soil-borne phytopathogenic fungi under *in vitro* conditions.

2 MATERIALS AND METHODS

2.1 Study objects and sample collection

The study materials consisted of water and sediment samples collected from the Rayim So‘fi hot spring located in Koson District, Kashkadarya Region, Uzbekistan, as well as rhizosphere soil samples obtained from cotton (*Gossypium hirsutum* L.) fields in Kasbi District. The samples were collected on May 19, 2026. At the time of sampling, the water temperature of the hot spring was 65°C, whereas the temperature of the cotton rhizosphere soil was 40°C. Water temperature at different locations within the hot spring was measured using a thermometer, while pH was determined using an indicator.

Three rhizosphere soil samples were collected from depths ranging from 10 to 45 cm along the root systems of cotton plants. Hot spring water and sediment samples were transferred into sterile, tightly sealed containers, while rhizosphere soil samples were placed in sterile polyethylene bags and transported to the laboratory. Until microbiological analysis, all samples were stored in a refrigerator at 4°C.

2.2 Isolation of thermophilic and thermotolerant bacteria

Thermophilic and thermotolerant bacteria were isolated from the collected environmental samples using serial tenfold dilution and spread-plating techniques. For this purpose, 1 mL of water sample or 1 g of soil or sediment sample was added to 9 mL of sterile physiological saline to obtain an initial suspension at a dilution of 10^{-1}. Subsequent serial tenfold dilutions were prepared up to 10^{-2}–10^{-6}. A volume of 0.1 mL from each dilution was spread evenly over the surface of solid growth media using a sterile spreader. The inoculated Petri dishes were incubated at temperatures of 45, 50, 55, 60, and 65°C. Bacterial colony development was monitored over a period of 24–72 h, while the incubation period was extended to 5–7 days for slowly growing microorganisms. The morphological characteristics of the resulting colonies were examined, and colonies differing in shape, size, color, surface texture, and margin characteristics were selected. The selected colonies were purified through repeated subculturing to obtain pure cultures. The ability of the bacterial

isolates to grow at different temperatures was used for the preliminary assessment of their thermophilic or thermotolerant characteristics.

2.3 Determination of the biochemical properties of bacterial isolates

The biochemical properties of the isolated bacterial strains were evaluated based on catalase activity and their ability to hydrolyze starch, casein, and lipid substrates. To determine enzymatic activities, culture media supplemented with the corresponding substrates were used, including Tryptic Soy Broth (TSB) and Starch Casein Agar (SCA). These enzymatic assays are commonly employed to characterize the phenotypic properties of bacterial isolates [13,14].

2.3.1 Catalase activity.

Catalase activity of the bacterial isolates was determined using a 3% hydrogen peroxide (H_2O_2) solution. A sample was collected from a freshly grown bacterial colony and placed on a clean glass microscope slide, followed by the addition of one drop of H_2O_2 solution. The formation of gas bubbles was considered a positive catalase reaction (+), whereas the absence of bubble formation was interpreted as a negative reaction (−) [13,15].

2.3.2 Amylolytic activity.

The amylolytic activity of the bacterial isolates was determined using a solid growth medium containing starch. The isolates were incubated at 50, 55, and 60°C for 5 days. Following incubation, Lugol's iodine solution was added to the surface of the culture medium. The formation of a clear halo zone around the bacterial colony was considered indicative of starch hydrolysis and recorded as a positive reaction (+), whereas the absence of a clear zone was recorded as a negative reaction (−) [13–15]

2.3.3 Proteolytic activity.

The proteolytic activity of the bacterial isolates was determined based on their ability to hydrolyze casein. The isolates were inoculated onto a solid culture medium containing casein and incubated at 50, 55, and 60°C for 5 days. The formation of a clear hydrolysis zone around the colonies was considered evidence of proteolytic activity and recorded as a positive reaction (+), whereas the absence of a hydrolysis zone was recorded as a negative reaction (−) [13,15].

2.3.4 Lipolytic activity.

The lipolytic activity of the bacterial isolates was evaluated using a culture medium supplemented with Tween 60 as a lipid substrate. The isolates were incubated at 50, 55, and 60°C for 7 days. Lipolytic activity was recorded as a positive (+) or negative (−) reaction based on visible changes in the medium surrounding the colonies associated with substrate hydrolysis [14].

All enzymatic assays were performed in triplicate. Uninoculated culture media served as negative controls. The results of the enzymatic assays were expressed qualitatively as positive (+) or negative (−) reactions.

2.4 Evaluation of antagonistic activity against phytopathogenic fungi

The antagonistic activity of thermophilic and thermotolerant bacterial isolates against phytopathogenic fungi was evaluated *in vitro* using the dual-culture assay [16].

The fungal pathogens used as indicator organisms in this study were *Fusarium oxysporum* f. sp. ciceri, *Fusarium oxysporum* f. sp. lycopersici, and *Rhizoctonia solani*. The phytopathogenic fungal cultures were maintained on Potato Dextrose Agar (PDA) medium. Mycelial plugs measuring 5–6 mm in diameter were excised from the actively growing margins of 5–7-day-old fungal colonies using a sterile cork borer. The bacterial isolates were pre-cultured on Nutrient Agar (NA) medium before the antagonistic assays.

In the dual-culture assays, a mycelial plug of the phytopathogenic fungus was placed on one side of a Petri dish containing PDA medium, while the bacterial isolate was inoculated on the opposite side. The experiments were conducted at approximately 27°C for 5–8 days. Each bacterial isolate–phytopathogen combination was tested in triplicate. In the control treatment, PDA medium inoculated only with the phytopathogenic fungus was used.

Antagonistic activity was evaluated based on the radial growth of fungal mycelia in the direction of the bacterial isolate. Radial growth distances were measured in millimeters in both control and experimental treatments. The percentage inhibition of phytopathogenic fungal mycelial growth was calculated using the following formula:

$$I(\%)=\frac{C-T}{C}\times100$$

Where: **I** – percentage inhibition of phytopathogenic fungal mycelial growth (%);

C – radial growth distance of fungal mycelia in the control treatment, measured in the direction corresponding to the bacterial inoculation point (mm);

T – radial growth distance of fungal mycelia in the presence of the bacterial antagonist, measured in the direction facing the bacterial isolate (mm).

The antagonistic activity of the bacterial isolates was evaluated based on the reduction in phytopathogenic fungal mycelial growth relative to the control treatment and the observation of growth inhibition between bacterial and fungal colonies.

2.5 Statistical analysis

The experimental data were statistically analyzed based on three replicates. Quantitative measurements were expressed as the arithmetic mean (M) and standard deviation (SD), presented as M ± SD. Antagonistic activity was calculated separately for each bacterial isolate–phytopathogen combination.

3 RESULTS AND DISCUSSION

3.1 Morphological characteristics of bacterial isolates obtained from natural sources

Among the bacterial isolates obtained from the Rayim So‘fi hot spring and cotton rhizosphere soil in Kashkadarya Region, two isolates capable of growing at elevated temperatures and exhibiting distinct morphological characteristics were selected for further investigation. Their colony morphology, cell shape, and Gram-staining characteristics were examined (Table 1).

Table 1: Morphological characteristics of bacterial isolates obtained from natural sources

Characteristics	Hot spring isolate	Cotton rhizosphere isolate
Preliminary identification	*Geobacillus* sp. B1	*Bacillus* sp. M11
Source	Rayim So‘fi hot spring water	Cotton rhizosphere soil
Colony color	Light yellow	Yellowish-cream
Colony shape and surface	Smooth, shiny, with entire margins	Circular, smooth, convex, with entire margins
Gram staining	Positive	Positive
Cell morphology	Rod-shaped	Rod-shaped
Cell arrangement	Single cells and chains	Single cells and short chains

The isolate obtained from the hot spring water formed light-yellow colonies on the culture medium, characterized by entire margins, a smooth surface, and a glossy appearance. Microscopic examination revealed that the cells were Gram-positive and exhibited straight or slightly elongated rod-shaped morphology. The cells occurred singly, in short chains, and occasionally in longer chains. Based on these morphological characteristics, the isolate was provisionally characterized as *Geobacillus* sp. B1.

The isolate obtained from cotton rhizosphere soil formed yellowish-cream, circular, smooth, convex colonies with entire margins. Microscopic examination revealed Gram-positive, rod-shaped cells occurring singly and in short chains. Based on these phenotypic characteristics, the isolate was provisionally characterized as *Bacillus* sp. M11 (Figure 1).

The morphological characteristics of the isolated bacterial strains were examined using pure cultures grown for 24 h. Colony shape, color, size, surface characteristics, and margin morphology were assessed macroscopically. Microscopic examination was performed to determine bacterial cell shape, size, and arrangement. Gram-staining characteristics and endospore formation were investigated following the methodological approaches described in *Bergey's Manual of Determinative Bacteriology* [17].

The results demonstrated that the bacterial isolates obtained from hot spring water and cotton rhizosphere soil differed in their colony morphology and cellular arrangement. Further identification studies are required to confirm the taxonomic affiliation of the isolates.

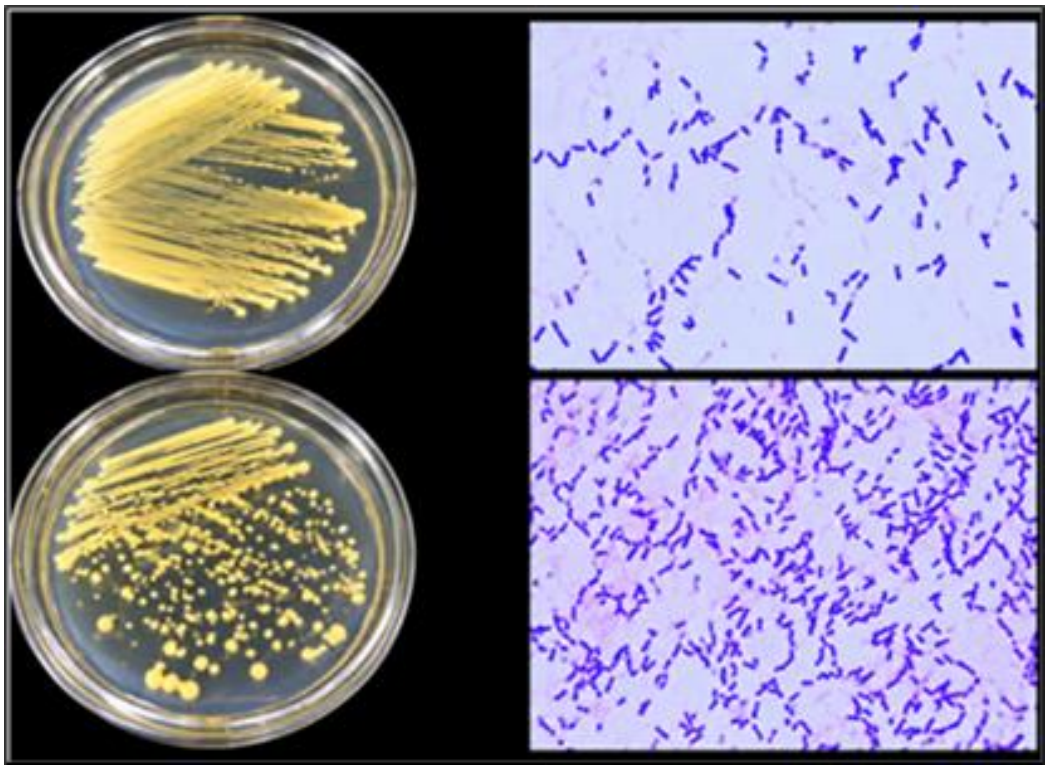


Figure 1: Colony and cellular morphology of bacterial isolates obtained from Rayim So‘fi hot spring water and cotton rhizosphere soil.

3.2 Biochemical characteristics of bacterial isolates

The enzymatic properties of the *Geobacillus* sp. B1 and *Bacillus* sp. M11 isolates obtained from natural sources were evaluated based on their ability to hydrolyze casein, starch, and lipid substrates, as well as their catalase activity (Table 2).

Table 2: Biochemical characteristics of the selected bacterial isolates

Biochemical property	*Geobacillus* sp. B1	*Bacillus* sp. M11
Starch hydrolysis (amylolytic activity)	+	+
Tween 60 hydrolysis (lipolytic activity)	–	–
Catalase activity	+++	+
Casein hydrolysis (proteolytic activity)	+	+

Note: (+) positive; (–) negative; (+++) strongly positive.

The results demonstrated that both bacterial isolates exhibited proteolytic and amylolytic activities. The formation of clear hydrolysis zones around the colonies on casein-containing culture medium indicated that both isolates were capable of hydrolyzing casein (Figure 2).



Figure 2: Casein hydrolysis by bacterial isolates on skim milk agar medium (A — *Geobacillus* sp. B1; B — *Bacillus* sp. M11).

Furthermore, the positive hydrolysis reactions observed on starch-containing culture medium confirmed the amylolytic activity of both isolates.

In the catalase assay, *Geobacillus* sp. B1 exhibited a strongly positive reaction (+++), whereas *Bacillus* sp. M11 showed a positive reaction (+). These findings indicate that both isolates possess catalase activity associated with the decomposition of hydrogen peroxide. However, the observed differences in reaction intensity alone are insufficient to establish differences in the quantitative activity of the catalase enzyme.

The lipolytic activity assay performed using Tween 60 as a substrate yielded negative results for both isolates. This finding indicates that lipolytic activity was not detectable under the experimental conditions employed.

Overall, the *Geobacillus* sp. B1 and *Bacillus* sp. M11 isolates demonstrated proteolytic, amylolytic, and catalase activities, indicating their capacity to produce certain hydrolytic enzymes. These findings contribute to the physiological and biochemical characterization of the isolates; however, they do not directly establish the mechanisms underlying their antagonistic effects against phytopathogenic fungi.

3.3 Antagonistic activity of bacterial isolates against phytopathogenic fungi

The antagonistic activity of the *Geobacillus* sp. B1 and *Bacillus* sp. M11 isolates obtained from natural sources against soil-borne phytopathogenic fungi was evaluated in vitro using the dual-culture assay. The indicator phytopathogens used in this study included *Fusarium oxysporum* f. sp. ciceri, F. oxysporum f. sp. lycopersici, and *Rhizoctonia solani*. All phytopathogenic fungal cultures were obtained from the collection of the Phytobiocontrol Laboratory of the Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan.

The co-growth of bacterial isolates and phytopathogenic fungi was monitored at 27°C for 5–8 days. In the control treatments, the phytopathogenic fungi were cultivated in the absence of bacterial antagonists. The dual-culture assay demonstrated that the presence of certain bacterial isolates restricted the mycelial growth of the phytopathogenic fungi (Figure 3).

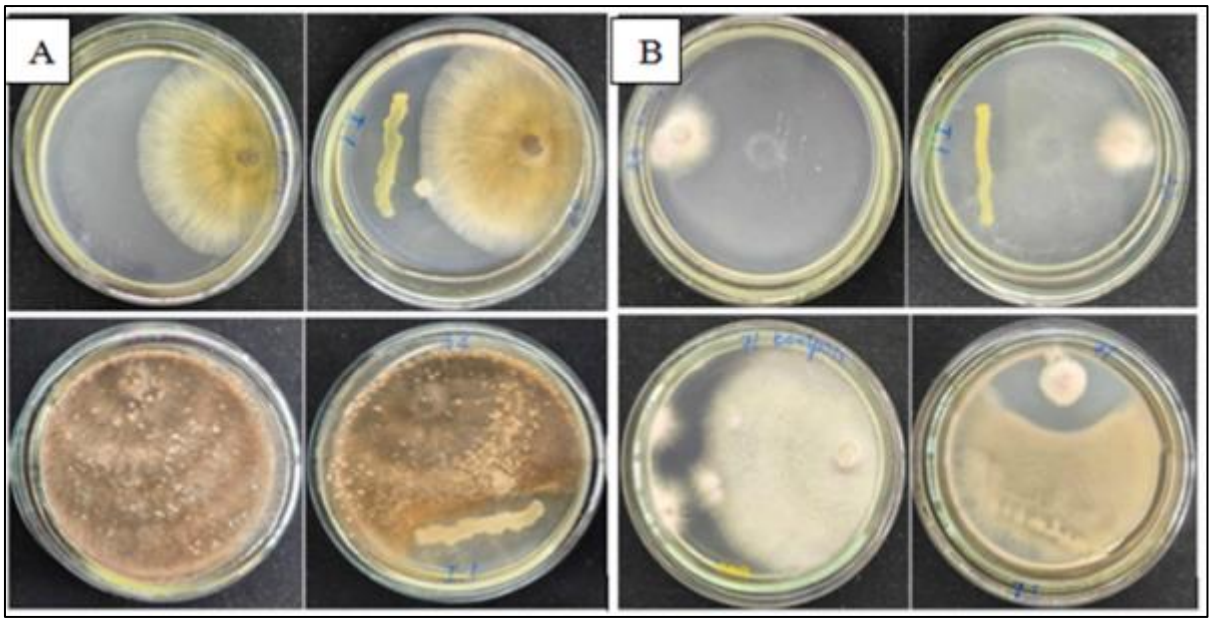


Figure 3: In vitro antagonistic activity of *Geobacillus* sp. B1 and *Bacillus* sp. M11 isolates against phytopathogenic fungi (A — *Rhizoctonia solani*; B — *Fusarium oxysporum* f. sp. *ciceri*).

The interaction between the hot spring-derived isolate *Geobacillus* sp. B1 and *F. oxysporum* f. sp. *ciceri* resulted in an inhibition value of 31.32%. In the assay involving *Bacillus* sp. M11, isolated from cotton rhizosphere soil, the corresponding value was 28.53%. The biological significance of these values depends on the parameter measured; therefore, their quantitative interpretation as antagonistic efficacy requires comparison with the corresponding control treatments.

Table 3: In vitro evaluation of the inhibitory effects of *Geobacillus* sp. B1 and *Bacillus* sp. M11 isolates on the mycelial growth of phytopathogenic fungi

Bacterial isolate	Phytopathogen	Radial growth in control (mm)	Radial growth in treatment (mm)	Inhibition (%)
Geobacillus sp. B1	*R. solani*	45.23	31.67	29.98
Geobacillus sp. B1	*F. oxysporum* f. sp. *ciceri*	35.44	24.34	31.32
Geobacillus sp. B1	*F. oxysporum* f. sp. *lycopersici*	36.34	27.52	24.27
Bacillus sp. M11	*R. solani*	47.12	28.33	39.88
Bacillus sp. M11	*F. oxysporum* f. sp. *ciceri*	27.23	19.46	28.53
Bacillus sp. M11	*F. oxysporum* f. sp. *lycopersici*	29.43	23.47	20.25

The growth of phytopathogenic fungal colonies was restricted in the direction of the bacterial antagonists in certain experimental treatments. This observation suggests that the selected isolates possess potential antagonistic properties against phytopathogenic fungi. However, reliable assessment of the degree of inhibition and differences between the isolates requires comparison of radial growth measurements in control and experimental treatments, supported by appropriate statistical analysis.

Overall, the preliminary *in vitro* investigations demonstrated that the bacterial isolates obtained from hot spring water and cotton rhizosphere soil include potential candidates capable of suppressing the mycelial growth of phytopathogenic

fungi. These findings provide a basis for further quantitative and mechanistic investigations of the antagonistic activities of the selected isolates.

4 DISCUSSION

The isolation of thermophilic and thermotolerant bacteria from natural sources and the investigation of their antagonistic properties against phytopathogenic fungi represent an important approach to identifying promising microorganisms for biological plant protection. In the present study, the morphological, physiological, biochemical, and antagonistic properties of two bacterial isolates obtained from the Rayim So'fi hot spring and cotton rhizosphere soil in Kashkadarya Region were evaluated. The selected isolates were Gram-positive and rod-shaped, and were provisionally characterized as *Geobacillus* sp. B1 and *Bacillus* sp. M11 based on their colony morphology and selected phenotypic characteristics. However, Gram-staining characteristics, cell morphology, and enzymatic reactions alone are insufficient to conclusively establish taxonomic affiliation at the genus or species level. Akanbi et al. (2010) and Panda et al. (2013) [18,19] also employed conventional morphological, physiological, and biochemical approaches to characterize thermophilic bacterial isolates. Although these methods are important for preliminary phenotypic characterization, contemporary taxonomic studies require their supplementation with molecular identification techniques. Therefore, the designations *Geobacillus* sp. B1 and *Bacillus* sp. M11 used in the present study should be interpreted as preliminary identification results.

The ability of bacterial isolates to grow at different temperatures is an important physiological indicator of their ecological adaptability. The growth of the hot spring-derived isolate under elevated-temperature conditions suggests its potential affiliation with the group of thermophilic microorganisms. In contrast, the ability of the isolate obtained from cotton rhizosphere soil to tolerate elevated temperatures indicates its thermotolerant characteristics. Nevertheless, accurate differentiation between thermophilic and thermotolerant microorganisms requires determination of not only their ability to grow at elevated temperatures but also their minimum, optimum, and maximum growth temperatures. In particular, when assessing the potential application of bacteria isolated from high-temperature natural environments, such as hot springs, under agricultural conditions, their ability to maintain metabolic activity at typical soil temperatures is of particular importance. This is because the growth of a microorganism under laboratory conditions at elevated temperatures does not necessarily imply its stable colonization of the plant rhizosphere or its effective control of phytopathogens.

In the present study, both isolates demonstrated the ability to hydrolyze casein and starch, indicating positive proteolytic and amylolytic activities. In the catalase assay, *Geobacillus* sp. B1 exhibited a strongly positive reaction (+++), whereas *Bacillus* sp. M11 showed a positive reaction (+). These findings indicate that both isolates possess the ability to decompose hydrogen peroxide. However, the visual intensity of the catalase reaction does not provide a reliable quantitative comparison of enzyme activity. In contrast, no lipolytic activity was detected in either isolate using Tween 60 as a substrate. This finding does not necessarily indicate that these bacteria are incapable of hydrolyzing all types of lipid substrates; rather, it demonstrates that the corresponding reaction was not detected under the culture and incubation conditions employed. Singh et al. [20] reported catalase activity and casein hydrolysis in *Bacillus cereus* strain SIU1 isolated from soils in Uttar Pradesh. These findings are consistent with certain biochemical characteristics observed in the present study. Nevertheless, similar enzymatic properties alone cannot independently confirm that bacterial isolates belong to the same taxonomic group.

Proteolytic activity reflects the ability of bacteria to degrade complex protein substrates, whereas amylolytic activity is associated with the hydrolysis of polysaccharides such as starch. These properties may expand the ability of bacteria to utilize organic compounds in their natural environments. However, positive results for casein and starch hydrolysis do not necessarily indicate that the isolates directly damage the cell wall or membrane of phytopathogenic fungi. Further investigations of chitinase, β-1,3-glucanase, and other hydrolytic enzymes targeting the major structural components of fungal cell walls are required. Previous studies have demonstrated that the effectiveness of *Bacillus* species in biological control is associated with several mechanisms, including the production of hydrolytic enzymes, antimicrobial metabolites, siderophores, and volatile organic compounds, as well as competition for nutrients and ecological niches [21]. From this perspective, although the proteolytic and amylolytic activities identified in the present study characterize the biochemical potential of the isolates, they are insufficient to determine the specific mechanisms underlying their antagonistic effects.

Studies investigating the activity of thermophilic and thermotolerant bacteria against phytopathogens have confirmed the practical significance of this research area. Ait Kaki et al. [22] investigated *Bacillus* isolates obtained from an environment associated with the Ouled Yalass hot spring in Algeria and reported high antagonistic activity of *B. amyloliquefaciens* strain 4RH against *Fusarium oxysporum* and *Botrytis cinerea*. The authors also characterized the proteolytic and cellulolytic activities of this strain, its ability to produce antimicrobial lipopeptides, and certain properties associated with plant growth promotion. Such comprehensive approaches demonstrate that the selection of

antagonistic microorganisms should not be limited to recording the inhibition of phytopathogen growth; the biological mechanisms underlying the observed effects should also be investigated.

Pawar and Borkar [23] investigated the antagonistic activity of thermophilic bacteria isolated from various environments, including boiled milk, compost, and tomato rhizosphere. The authors reported the antagonistic effects of isolates identified as *Bacillus licheniformis* and *Bacillus stearothermophilus* (according to the historical nomenclature) against *Rhizoctonia bataticola*, *R. solani*, and *Pythium aphanidermatum*. These findings indicate that thermophilic bacteria may occur not only in geothermal environments but also in organic matter-rich habitats and plant-associated ecological niches, where they may exhibit potential activity against phytopathogens. However, direct quantitative comparisons between studies are limited by differences in the phytopathogenic strains, culture media, incubation temperatures, and criteria used to assess antagonistic activity.

In the present study, the antagonistic properties of *Geobacillus* sp. B1 and *Bacillus* sp. M11 isolates against *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. ciceri, and *F. oxysporum* f. sp. lycopersici were investigated using the dual-culture assay. In certain bacterium–phytopathogen combinations, restricted mycelial growth was observed in the direction of the bacterial antagonist. In the experiment involving the hot spring isolate and R. solani, a value of 31.67 mm was recorded, whereas a value of 28.33 mm was obtained in the experiment involving the cotton rhizosphere isolate and R. solani. However, these measurements represent treated radial growth distances and must be interpreted relative to the corresponding control measurements. Furthermore, it would not be scientifically justified to conclude that one isolate was more effective than the other based solely on these two values, since they were obtained from experiments involving different phytopathogens.

Singh et al. [24] investigated the antagonistic properties of the thermotolerant marine-derived strain *Bacillus amyloliquefaciens* S185 against *F. oxysporum* f. sp. *cubense* and reported 78% inhibition of mycelial growth in a dual-culture assay. The strain was capable of growing at temperatures ranging from 20 to 50°C. The authors isolated an antifungal compound and characterized it using high-resolution mass spectrometry and nuclear magnetic resonance (NMR) analyses, identifying it as iturin A5. The study demonstrated that this compound adversely affects the germination of phytopathogenic fungal spores and hyphal development. These findings illustrate that the biological mechanisms underlying antagonistic activity can be more thoroughly elucidated by linking initial phenotypic observations of antagonism with the chemical identification of microbial metabolites.

The antifungal activity of *Bacillus* species is frequently associated with cyclic lipopeptides belonging to the iturin, fengycin, and surfactin families. Fira et al. [21] reviewed the biological control mechanisms employed by *Bacillus* bacteria against phytopathogens and demonstrated the importance of secondary metabolite production, competition with pathogens, and activation of plant defense responses. Subsequent studies have also emphasized that lipopeptides can affect the integrity of fungal cell membranes and their physiological processes, while the combined effects of different bioactive compounds may determine the overall antagonistic efficacy. Nevertheless, the presence of iturin, fengycin, surfactin, or other antifungal metabolites was not investigated in the present study. Therefore, attributing the observed antagonistic activity specifically to the production of these compounds can currently be considered only a scientific hypothesis.

Other studies have also investigated bacteria isolated from extreme environments as potential biological control agents. In particular, bacterial isolates obtained from the geothermal regions of the Atacama Desert, the thermal environment of Lake Bogoria in Kenya, and the Jakrem and Garampani hot springs have been investigated for their hydrolytic enzyme activities, plant growth-promoting properties, and antagonistic effects against phytopathogens [25]. Some studies have employed additional approaches, including molecular identification, secondary metabolite analysis, and electron microscopy. These investigations demonstrate the considerable potential of geothermal environments as sources of biologically active microorganisms[26,27]. However, the isolation of bacteria from such environments does not automatically guarantee high antagonistic efficacy or stable performance under agricultural conditions.

One of the important aspects of the present study is the combined assessment of the high-temperature adaptability, hydrolytic enzymatic properties, and preliminary antagonistic activity against phytopathogens of two bacterial isolates obtained from different ecological sources. Of particular importance is the fact that the antagonistic experiments were conducted at approximately 27°C. The ability of bacteria isolated from high-temperature environments to inhibit phytopathogen growth under standard laboratory temperature conditions provides a basis for considering them as potential candidates for further biocontrol investigations. Nevertheless, further studies are required to determine the optimal growth temperatures of the bacterial isolates, confirm their taxonomic affiliations using molecular methods, calculate antagonistic activity as a percentage relative to control treatments, and statistically evaluate the results. In addition, investigating the antifungal activity of cell-free culture filtrates, chitinolytic and glucanolytic enzymes, secondary metabolites, and the ability to colonize plant rhizospheres would help elucidate the mechanisms underlying the observed effects.

Overall, the obtained results indicate that the Rayim So‘fi hot spring and cotton rhizosphere soil represent promising natural sources for the isolation of bacteria adapted to elevated temperatures and exhibiting antagonistic activity against certain phytopathogenic fungi. The detection of proteolytic, amylolytic, and catalase activities in the isolates provisionally designated as *Geobacillus* sp. B1 and *Bacillus* sp. M11 characterizes their biochemical properties, while the inhibition of phytopathogenic fungal mycelial growth under *in vitro* conditions provides a basis for considering them as candidates for further biological control studies. However, these findings do not yet establish their effectiveness as practical biopreparation agents. Further investigations involving reproducible quantitative antagonistic assays, safety assessments, greenhouse experiments using plant–pathogen–antagonist systems, and subsequent field trials are required.

5 CONCLUSION

In this study, the morphological, physiological, biochemical, and antagonistic properties of thermophilic and thermotolerant bacterial isolates obtained from water samples of the Rayim So‘fi hot spring and cotton rhizosphere soil in the Kashkadarya region were investigated. The two selected isolates exhibited Gram-positive, rod-shaped cell morphology and were provisionally characterized as *Geobacillus* sp. B1 and *Bacillus* sp. M11 based on their phenotypic characteristics. Both isolates demonstrated casein and starch hydrolysis, as well as catalase activity, whereas no lipolytic activity was observed in the presence of the Tween 60 substrate. Antagonistic assays conducted under in vitro conditions revealed that the bacterial isolates were capable of restricting the mycelial growth of certain phytopathogenic fungi. Specifically, inhibition values of 31.32% and 28.53% were recorded for the interactions of *Geobacillus* sp. B1 and *Bacillus* sp. M11 with. *F oxysporum* f. sp. ciceri, respectively. The obtained results indicate that hot spring water and cotton rhizosphere soil are promising ecological sources for the isolation of bacteria adapted to elevated temperatures and exhibiting antagonistic properties. The isolated strains were therefore considered potential candidates for further research aimed at developing biological control agents against phytopathogenic fungi.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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