

Article

Harnessing Extremophile *Bacillus* spp. for Biocontrol of *Fusarium solani* in *Phaseolus vulgaris* L. Agroecosystems

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Abstract

Common bean (*Phaseolus vulgaris* L.) is a critical protein-rich legume supporting food and nutritional security globally. However, *Fusarium* wilt, caused by *Fusarium solani*, remains a major constraint to production, with yield losses reaching up to 84%. While biocontrol strategies have been explored, most microbial agents are sourced from mesophilic environments and show limited effectiveness under abiotic stress. Here, we report the isolation and characterization of extremophilic *Bacillus* spp. from the hypersaline Lake Bogoria, Kenya, and their biocontrol potential against *F. solani*. From 30 isolates obtained via serial dilution, 9 exhibited antagonistic activity in vitro, with mycelial inhibition ranging from 1.07–1.93 cm. 16S rRNA sequencing revealed taxonomic diversity within the *Bacillus* genus, including unique extremotolerant strains. Molecular screening identified genes associated with the biosynthesis of antifungal metabolites such as 2,4-diacetylphloroglucinol, pyrrolnitrin, and hydrogen cyanide. Enzyme assays confirmed substantial production of chitinase (1.33–3160 U/mL) and chitosanase (10.62–28.33 mm), supporting a cell wall-targeted antagonism mechanism. In planta assays with the lead isolate (B7) significantly reduced disease incidence (8–35%) and wilt severity (1–5 affected plants), while enhancing root colonization under pathogen pressure. These findings demonstrate that extremophile-derived *Bacillus* spp. possess robust antifungal traits and highlight their potential as climate-resilient biocontrol agents for sustainable bean production in arid and semi-arid agroecosystems.

Keywords: antibiosis genes; biocontrol; *Fusarium solani*; *Phaseolus vulgaris* L.; *Bacillus* spp.; soda lake



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1. Introduction

In Kenya, Agriculture remains a foundational pillar of Kenya's economy, contributing approximately 21.8% to the national GDP [1]. Within this sector, vegetable production plays a vital role in promoting food security and sustaining the livelihoods of smallholder farmers. Among the key vegetable crops, the common bean (*Phaseolus vulgaris* L.) is of particular dietary and economic importance. Known for its edible dry seeds and green pods [2], it is the second most consumed staple food in Kenya after maize, valued for its nutritional benefits, especially its high protein content and contribution to dietary diversity [3].

Beyond the seeds and pods, common bean leaves are occasionally utilized as leafy vegetables or livestock fodder [4]. Nutritionally, the crop is a rich source of protein, dietary fiber, thiamine, folate, and essential minerals such as iron and zinc nutrients

commonly deficient in cereal- and root-based diets [1,2]. Additionally, common beans contain phytochemicals with analgesic and neuroprotective properties, further enhancing their health benefits [1]. Their high fiber content supports gastrointestinal health, stabilizes blood glucose levels, and promotes satiety, thereby aiding in weight management [5].

From an economic standpoint, common beans constitute a vital cash crop for Kenya's smallholder farmers [6], contributing significantly to household incomes and rural economic development [7]. The beans are also exported to various international markets, where their distinctive flavor and quality have sustained high demand [7]. However, domestic production frequently falls short of meeting this demand, leading to substantial imports from neighboring countries, including Uganda, Ethiopia, and Tanzania [1].

This reliance on imports has coincided with heightened pest and disease incidence in bean production systems [8]. Major insect pests include cutworms, bean flies, aphids, pod borers, whiteflies, red spider mites, and thrips, while important diseases affecting the crop comprise black root rot, damping-off, bean rust, *Fusarium* wilt, bacterial blight, and downy mildew [9].

Soil infertility and increasing environmental stress have compounded these challenges, contributing to a 13.8% decline in bean yields over the past five years [10]. Particularly devastating is root and stem rot, which affects between 50% and 70% of bean crops and is predominantly caused by *Fusarium solani* [11]. This soil-dwelling fungus poses a significant challenge due to its capacity to persist as chlamydospores, colonize decaying organic matter, and spread via runoff and contaminated plant residues [12].

Symptoms of infection include irregular, light-brown lesions on the stem, taproot, and hypocotyl, which progressively darken and enlarge [13,14]. The pathogen typically enters through root wounds or natural openings, resulting in systemic infections that can cause yield losses of up to 70% [15,16]. These severe impacts underscore the pressing need for more effective and sustainable disease management strategies.

Control approaches for *F. solani* have included both chemical and cultural methods. While seed treatment with synthetic fungicides such as prochloraz, bromuconazole, and benomyl has shown efficacy against related *Fusarium* species [17], concerns persist regarding fungicide resistance and adverse effects on non-target organisms. As a result, there has been a growing emphasis on integrated cultural practices, including crop rotation, improved sanitation, and the use of genetically diverse cultivars, to reduce disease pressure and enhance soil resilience [18,19]. Other approaches include adjusting the sowing density and timing, selecting appropriate soil types [20], using resistant varieties or species mixtures [21], and practicing selective weeding [22].

Biological control offers a promising and sustainable alternative to conventional disease management approaches, including synthetic fungicides. It aligns well with environmentally conscious and pesticide-free agricultural systems. Among the most effective biocontrol candidates are strains from the *Bacillus* genus, which have demonstrated strong antagonistic activity against *Fusarium* species. Notably, *Bacillus velezensis* has shown potential in suppressing *Fusarium solani* [23], while *Bacillus subtilis* strain CAS 32 has been reported to effectively control *F. solani*-induced root rot in common beans [24]. Overall, members of the *Bacillus* genus are widely recognized for their capacity to mitigate *Fusarium*-related diseases in various crops [25]. Additionally, the study by Guevara-Avendaño et al. [26] reported that *Bacillus* spp. strains enhance the suppression of *Fusarium* wilt in radish. The antagonistic potential of *Bacillus* species against *Fusarium solani* has been widely explored to evaluate their effectiveness as biocontrol agents and to gain insight into the underlying mechanisms of suppression [27]. Both in vitro and in vivo experiments have been employed to assess the ability of *Bacillus* strains to inhibit *F. solani* and other pathogenic fungi, highlighting their broad-spectrum antifungal properties plant-pathogen interactions.

These antagonistic effects are mediated through multiple mechanisms, including mycoparasitism, induction of systemic resistance in host plants, secretion of inhibitory secondary metabolites, stimulation of plant growth, competition for nutrients and ecological niches, and the production of cell wall-degrading lytic enzymes [27–30]. An emerging frontier in plant disease management involves exploring the microbial communities inhabiting Kenya’s extreme environments, particularly its soda lakes—Bogoria, Magadi, and Elementaita. These alkaline, saline ecosystems are known to host diverse extremophilic bacteria, as demonstrated by multiple studies investigating microbial populations in lake sediments, waters, and surrounding soils [31–34]. In a related investigation, Mutungi et al. [35] identified endophytic microbes associated with shrub vegetation along the receding shores of Lake Bogoria, targeting their potential to suppress *Fusarium solani*, a major pathogen of common beans in Kenya. These findings underscore the biocontrol potential of extremophilic bacteria from soda lakes in mitigating bean diseases. Among the promising candidates is *Bacillus tequilensis*, an extremophile isolated from the hypersaline environment of Lake Bogoria. This species exhibits both remarkable salt tolerance and broad-spectrum antifungal activity, making it a strong candidate for the development of sustainable, environmentally friendly plant protection strategies. Accordingly, the present study focused on isolating and evaluating *Bacillus* spp. from Lake Bogoria through both in vitro and in vivo assays to assess their efficacy as biocontrol agents against *F. solani* in common bean (*Phaseolus vulgaris*) production.

2. Materials and Methods

2.1. Description of the Sampling Site and Sample Collection

Lake Bogoria, located in Baringo County, Kenya, is a saline-alkaline lake recognized as a Ramsar site and designated as a national reserve since 2 November 1973. The lake is characterized by its high salinity, geothermal activity, and numerous hot springs and geysers along its shores, with temperatures exceeding 45 °C. It is relatively shallow, measuring approximately 34 km in length and 3.5 km in width. The lake’s waters are rich in sodium (Na^+), bicarbonate (HCO_3^- ; 34,950 mg/L), and carbonate (CO_3^{2-} ; 12,700 mg/L) ions, with a highly alkaline pH above 8.0 and temperatures ranging from 39.0 to 98.5 °C. For this study, purposive sampling was conducted at six distinct geothermal sites (hot springs) around the lake. From each location, soil samples (100 g) were collected in triplicate, and the corresponding physicochemical parameters were recorded (Table 1). The samples were placed in sterile tubes, stored in a cool box, and transported to the Jomo Kenyatta University of Agriculture and Technology (JKUAT) for subsequent laboratory analysis.

Table 1. Sampling points with their physicochemical characteristics.

Sampling Point	Latitude	Longitude	Elevation (m)	pH	Conductivity (mS/cm)	Salt (ppt)	TDS (ppt)
BP01	N 10°43.692	E 058°25.621	1009	9.6	6.0	2.9	28.6
BP02	N 00°13.730	E 036°05.572	1009	9.2	25.6	12.1	17.0
BP03	N 00°13.711	E 036°05.575	1010	9.6	10.0	10.1	13.4
BP04	N 00°13.716	E 036°05.577	1008	8.8	6.1	2.9	3.9
BP05	N 00°13.724	E 036°05.584	1007	8.7	5.9	3.1	4.1
BP06	N 00°13.673	E 036°05.603	1007	8.2	26.1	13.0	17.2

Note: TDS—Total Dissolved Solids; BP—Bogoria point; M—Meter; pH—Potential of Hydrogen.

2.2. Isolation of *Bacillus* spp.

The isolation of *Bacillus* species from soil samples was carried out following the protocol described by Atlas [36], with slight modifications. Briefly, 1 g of each soil sample was suspended in 9 mL of sterile physiological saline solution (0.85% NaCl) and thoroughly

homogenized. The resulting suspension was serially diluted (1:9) up to a 10^{-4} dilution. From each dilution, 30 μ L aliquots were plated onto Nutrient Agar (HiMedia, Mumbai, India) and incubated at 30 °C for 48 h. Colonies exhibiting morphological characteristics typical of *Bacillus* spp. and showing bluish-green fluorescence under UV light (254 nm) were selected, sub-cultured for purification, and preserved for further characterization.

2.3. Antibiosis Assay of *Bacillus* spp. Isolates Against *Fusarium solani*

The antagonistic activity of *Bacillus* spp. isolates against *Fusarium solani* was evaluated using the dual culture technique, following the method described by Aydi et al. [37]. The assay was conducted on Sabouraud Dextrose Agar (SDA; HiMedia) composed of 10.0 g/L peptones, 40.0 g/L dextrose monohydrate, and 15.0 g/L agar, poured into 90 mm Petri dishes. Bacterial suspensions (10^8 CFU/mL) were streaked in a straight line across one side of the plate, while an 8 mm diameter mycelial plug of *F. solani* (ATCC 11712), actively cultured for five days, was placed on the opposite side. The fungal strain was previously isolated from infected common beans and obtained from Dudutech | Bioline Agrosiences Africa (Naivasha, Kenya). Control plates containing only the fungal plug at the center were also prepared. All assays were performed in triplicate, and plates were incubated at 30 °C for 14 days. The extent of fungal growth was determined by measuring the length of mycelial expansion in centimeters using a ruler with 1 mm precision.

2.4. Molecular Characterization of the Selected Bacterial Isolates

Genomic DNA was extracted from the two bacterial isolates exhibiting the strongest antagonistic activity against *Fusarium solani* using the Bacterial Genomic DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada), following the manufacturer's protocol. DNA quality and quantity were assessed using the Qubit 4 Fluorometer (Q33238), yielding an A260/280 ratio of 1.8. The DNA concentration was standardized to 100 ng/ μ L. Amplification of the 16S rRNA gene was carried out using the universal primers fD1 and rP1. PCR reactions were prepared in a total volume of 40 μ L, comprising 20 μ L of master mix, 18.2 μ L of nuclease-free water, 0.4 μ L each of fD1 and rP1 primers (10 pmol), and 1.0 μ L of genomic DNA (10 ng/ μ L). Thermocycling was conducted using a Primus 96 thermocycler (PEQLAB, Erlangen, Germany) under conditions described previously by Wekesa et al. [23]. The PCR amplicons were then purified using the QIAquick PCR amplification kit (Qiagen, Hilden, Germany) and sent to Macrogen (Seoul, Republic of Korea) for sequencing.

Phylogenetic Analysis

The Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) site was used to compare the 16S rRNA of the bacterial isolates. The BLAST findings selected 16S rRNA gene sequences with the highest similarity index. The sequences were aligned using CLUSTAL W 2.0. and incorporated in MEGA 11 for pairwise and multiple sequence alignment [38]. Phylogenetic analysis was conducted using MEGA version 11, where the Kimura-2 parameter model was used to calculate the evolutionary distances and construct the phylogeny tree [38]. The evolutionary distance was calculated using the maximum composite likelihood technique. A bootstrap analysis of 1000 replicates denoted the evolutionary history of the already analyzed taxa.

2.5. Inoculum Preparation and Confrontation Assay

2.5.1. Inoculum Preparation

The bacterial strain B7 (1.0×10^{11} CFU/mL) and *Fusarium solani* (1.0×10^8 spores/mL) were employed to investigate bacterial–fungal interactions. Strain B7, which demonstrated the highest mycelial growth inhibition in dual culture assays, was initially cultured in Luria-Bertani (LB) broth (HiMedia; 10.0 g/L tryptone, 5.0 g/L yeast extract, 10.0 g/L NaCl)

at 30 °C in a shaker incubator set at 120 rpm for 24 h. Subsequently, 0.5 mL of this culture was transferred to 50 mL of half-strength LB broth and incubated under the same conditions. The resulting bacterial suspension was used as inoculum for confrontation experiments.

The *F. solani* isolate, previously obtained from Dudutech Laboratory, had been confirmed through both morphological and molecular characterization. Its pathogenicity has been validated in earlier studies [39]. For this study, the isolate was sub-cultured on Sabouraud Dextrose Agar (SDA; HiMedia) at 28 °C for seven days. Spores were harvested following the method described by Chakravarthi et al. [40] and used as inoculum to assess the mycophagous activity of strain B7.

2.5.2. Confrontation Assay/Mycophagous Activity

The mycophagous interaction between strain B7 and *Fusarium solani* was evaluated to assess the bacterium's ability to grow at the expense of the phytopathogen during direct contact. A confrontation assay was conducted using three treatments: T1—B7 inoculum alone, T2—*F. solani* inoculum alone (serving as the control), and T3—a combination of B7 and *F. solani*, following the method described by Báez-Astorga et al. [41]. All treatments were incubated at 30 °C in a shaking incubator at 120 rpm. At 12-, 24-, and 48-h post-inoculation, 200 µL aliquots were collected from each treatment to observe bacterial–fungal interactions under a light microscope, as previously described [41].

From each aliquot, 20 µL was placed onto a microscope slide, heat-fixed, and stained with 0.5% safranin for 30 s. Excess stain was removed by rinsing with distilled water, and the slides were air-dried. Microscopic examination was performed at 40× magnification, analyzing three random fields per replicate. The number of fungal hyphal segments was quantified for both the B7 + *F. solani* treatment and the *F. solani*-only control to determine the extent of bacterial colonization and interaction.

2.6. Screening for Genes Associated with Antibiosis

To determine the antibiotic genes responsible for antagonism, B7 bacteria were examined by PCR for the presence or absence of genes associated with antibiosis using specific gene primers (Table 2). Genes selected were coded for 2,4-diacetyl phloroglucinol (2,4-DAPG), pyrrolnitrin, phenazine-1-carboxylic acid, pyoluteorin, and hydrogen cyanide antibiotics. The primers were set in a PCR mixture with a total reaction volume of 25.0 µL consisting of 10.0 µL of master mix, 8.2 µL of PCR water, (10 ppm), 0.4 µL of the F primer, (10 ppm) of 0.4 µL of the R primer, and 1.0 µL of template DNA (10.0 ng/mL). The conditions of the PCR amplification cycle were initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 68 °C/62 °C (Table 2) for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The presence or absence of the PCR amplicon was checked by electrophoresis (100 V for 45 min) in 1% agarose gels stained with ethidium bromide for band visualization using a Digi Doc imaging system (UVP, Cambridge, UK).

Table 2. Target antibiosis genes that encode enzymes for the biosynthesis of various antibiotics in the B7 strain.

Target Genes	Primer	Sequences	Annealing	Expected PCR Product Size	References
phlD (2,4-DAPG)	Phl2a Phl2b	GAGGACGTCGAAGACCACCA ACCGCAGCATCGTGTATGAG	62 °C	745	[42]
phzCD (phenazine-1-carboxylic acid)	PCA2a PCA3b	TTGCCAAGCCTCGCTCCAAC CCGCGTTGTTCCCTCGTTCAT	68 °C	1150	[42]
prnD (pyrrolnitrin)	PRND1 PRND2	GGGGCGGGCCGTTGGTGATGGA YCCCGCSGCCTGYCTGGTCTG	68 °C	786	[43]

Table 2. Cont.

Target Genes	Primer	Sequences	Annealing	Expected PCR Product Size	References
pltC (pyoluteorin)	PLTC1	AACAGATCGCCCCGGTACAGAACG	68 °C	438	[43]
	PLTC2	AGGCCCGGACACTCAAGAACTCG			
pltB (pyoluteorin)	PltBf	CGGAGCATGGACCCCGAGC	68 °C	791	[44]
	PltBr	GTGCCCGATATTGGTCTTGACC			
hcnBC (hydrogen cyanide)	Aca	ACTGCCAGGGGCGGATGTGC	62 °C	587	[45]
	Acb	ACGATGTGCTCGGCGTAC			

2.7. Production of Lytic Enzyme Activity

2.7.1. Chitinase Activity

The chitinolytic activity of strain B7 was assessed using a fluorometric chitinase assay kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturer's protocol. One unit of chitinase activity was defined as the amount of enzyme required to liberate 1 μmol of 4-methylumbelliferone per minute from the substrate. To further characterize enzymatic specificity, both endochitinase and exochitinase activities were evaluated using distinct substrates provided in the assay kit. Exochitinase activity was measured using *N*-acetyl- β -D-glucosaminide (GlcNAc) and *N,N'*-diacetyl- β -D-chitobioside (GlcNAc₂), while endochitinase activity was assessed with β -D-*N,N',N''*-triacetylchitotriose (GlcNAc₃). All assays were performed in technical replicates to ensure accuracy and reproducibility.

2.7.2. Chitosanase Activity

The production of chitosanase by strain B7 was evaluated using chitosanase detection agar (CDA), following the method described by Fawzya and Chasanah [46]. Briefly, bacterial suspensions (1.0×10^8 CFU/mL) were applied onto sterile 6 mm white paper filter discs and allowed to air-dry. Discs inoculated with distilled water served as negative controls. Each disc was then placed on CDA plates (three discs per plate), and the plates were incubated at 28 °C for 48 h. Chitosanase activity was assessed by the formation of clear zones surrounding the bacterial discs, indicative of enzymatic degradation. The diameters of the clearance zones were measured using a ruler with 1 mm precision and recorded in centimeters.

2.8. In Vivo Antagonistic Potential of Selected B7. Using Pot Assays

An in vivo assay was conducted to assess the biocontrol efficacy of strain B7 against *Fusarium solani*-induced root rot and to monitor its concentration and persistence in the rhizosphere of common bean (*Phaseolus vulgaris*) under greenhouse conditions. The experiment employed a pot culture method as described by Wekesa et al. [47]. The bacterial inoculum was prepared by culturing strain B7 in 100 mL of nutrient broth (HiMedia) at 28 °C for 36 h in a shaker incubator at 120 rpm, and subsequently adjusted to a final concentration of 1.0×10^9 CFU/mL. The *F. solani* inoculum was generated by culturing the fungus in 250 mL of potato dextrose broth (PDB; HiMedia) at 28 °C for 14 days and standardized to 2.0×10^8 spores/mL. Common bean seeds (yellow variety) were surface sterilized by immersion in 70% ethanol for 30 s, followed by 5% sodium hypochlorite for 5 min, and rinsed three times with sterile distilled water. Seeds were sown (three seeds per pot) in pots (28 × 40 cm) containing 200 g of sterilized cocopeat substrate and maintained in a greenhouse for 35 days at temperatures ranging from 18–29 °C and relative humidity between 40–50%.

The experimental design followed a completely randomized block format with three replicates per treatment. The treatments were as follows: T1—*F. solani* inoculated five days prior to B7 application; T2—*F. solani* alone; T3—B7 alone; and T4—untreated control (no pathogen or bacteria). For treatments involving B7, the bacteria were applied

at three intervals: 7, 14, and 28 days post-sowing, directly at the root zone. In the fungal infection protocol, bean seedlings were inoculated by immersing their roots for 2 h in *F. solani* spore suspension (2.0×10^8 spores/mL). Disease severity was scored weekly for three weeks using a 1–7 disease rating scale as described by Bock et al. [48], with plants categorized as wilted or unwilted. The number of symptomatic plants was recorded for each treatment.

2.9. Survival and Population Density of the B7 Strain in Common Bean Roots

The survival and population density of the inoculated bacterial strain were monitored following the method described by Sunyar [49]. Root samples were collected weekly from each treatment group and submerged in sterile distilled water (10 mL per 10 mg of root tissue). The samples were incubated in a rotary shaker for 2 h to dislodge rhizosphere-associated bacteria. From each suspension, a 200 µL aliquot was serially diluted up to 10^{10} -fold (1:9 dilution ratio). Subsequently, 30 µL of the appropriate dilution was plated onto Nutrient Agar (HiMedia) and incubated at 25 °C for 48 h. Colony-forming units (CFUs) were enumerated under UV light to quantify bacterial populations.

2.10. Data Analysis

The raw data from antagonistic and confrontation assays and the production of lytic enzymes were subjected to a normality test using the Shapiro–Wilk test to evaluate the normal distribution. The data were also subjected to a homogeneity of variance test (Levene’s test) to check the assumption of equal variances across the data. The data were then subjected to Analysis of Variance (ANOVA) based on a linear model:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

whereby the following:

Y_{ij} is the observed response;

μ is the overall mean;

τ_i is the effect of the i -th treatment (e.g., biocontrol strain or condition);

ε_{ij} is the random error associated with the j -th observation in the i -th treatment group, assumed to be normally distributed with mean zero and constant variance.

to determine statistically significant differences at a threshold of $p < 0.05$. For means separation, the Least Significant Difference (LSD) test was used as a post hoc analysis using SAS version 8.0 software. Graphical presentation was performed using GraphPad Prism 6.

3. Results

3.1. Isolation and Antibiosis Assay

The isolated bacterial colonies from Lake Bogoria soil samples were used in preliminary dual culture tests with *F. solani*. A total of 30 bacterial isolates were isolated from the different sampling sites of L. Bogoria. Among the 30 bacteria tested, 9 bacterial strains showed a varied zone of inhibition (Table 3; Figure 1). Of the nine bacteria, two bacteria (B7- 1.83 ± 0.09^{ab} and B21- 1.93 ± 0.07^a) had the highest < 40% inhibition rate (Table 3; Figure 1).

Table 3. Antagonistic activity of bacteria isolated against *F. solani*.

Lakes	Isolate Code	Zone of Inhibition (cm)
Bogoria	Control	0.00 ± 0.00 ^g
	B21	1.93 ± 0.07 ^a
	B7	1.83 ± 0.09 ^{ab}
	B38	1.77 ± 0.15 ^{bc}
	B11	1.70 ± 0.06 ^{cd}
	B29	1.67 ± 0.07 ^{cd}
	B30	1.60 ± 0.06 ^{de}
	B17	1.53 ± 0.12 ^e
	B12	1.13 ± 0.09 ^f
	B19	1.07 ± 0.03 ^f
CV		22.40
F-Value		288.60
LSD		0.10
<i>p</i> >		0.05

The values indicate that the mean number of mycelium length from three biological replicates ($n = 3$) with the same superscript letter(s) within the same column is not significantly different according to Fisher's test. According to Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d, e, f, g) indicate significantly different means within a column. Data are presented as mean ± standard error.

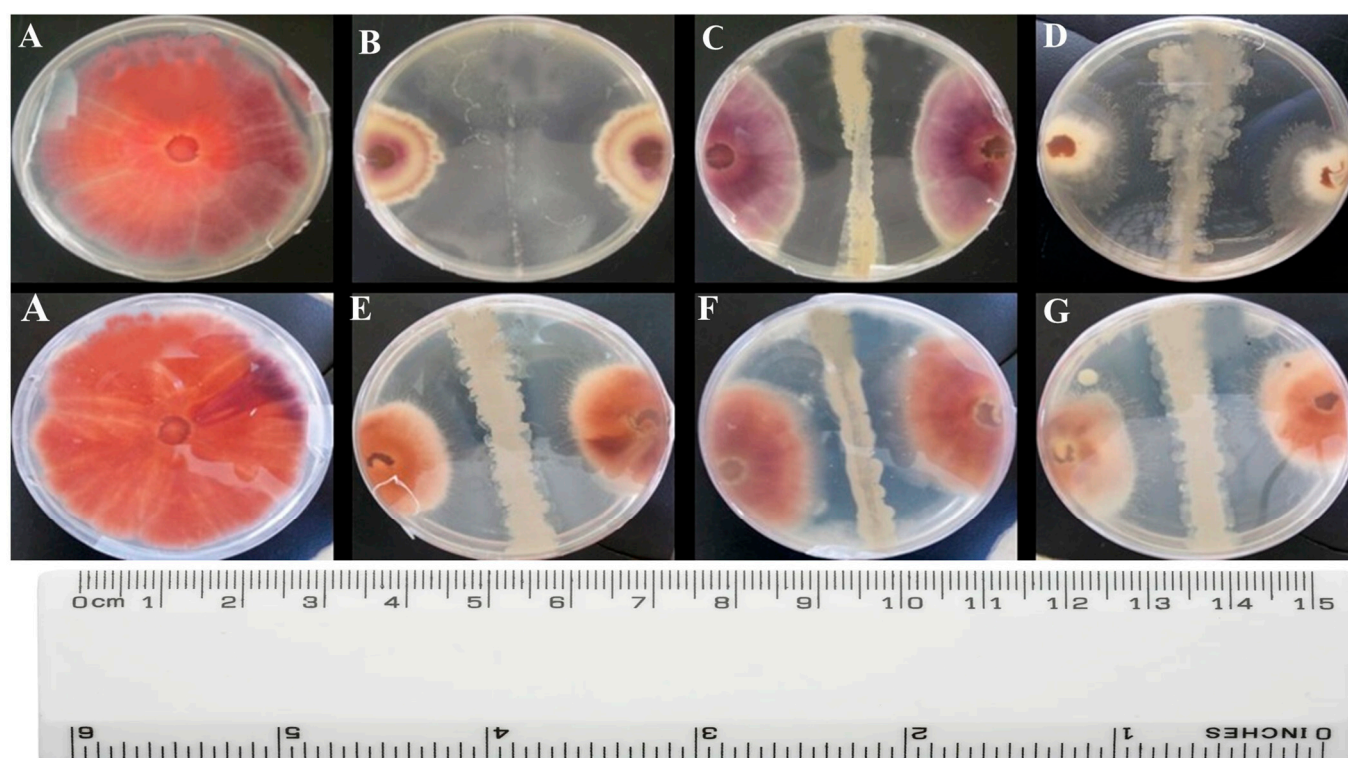


Figure 1. Antibiosis assay of positive Lake Bogoria bacterial isolates against *F. solani* after 14 days of incubation at 30° C ± 2.0: (A) *F. solani*-only, (B) B30 and *F. solani*, (C) B29 and *F. solani*, (D) B21 and *F. solani*, (E) B38 and *F. solani*, (F) B11 and *F. solani*, and (G) B7 and *F. solani*.

3.2. Molecular Characterization of Selected Bacteria Isolates

Sequencing was performed on only two bacteria isolates with the highest > 40.0% inhibition rate from the dual culture technique. From the partial sequence, the BLASTn analysis showed that two isolates belong to the genus *Bacillus* (Table 4; Figure 2). The two isolates were also submitted to the NCBI GenBank and assigned accession numbers PP331233 and PP331234, respectively (Figure 2). The bacteria strain B7 was closely related to the *Bacillus tequilensis* strain IKAK46 with a percentage identity of 100% (Figure 2).

However, the bacterial strain B21 was closely related to the strain of *Bacillus subtilis* subsp. *Inaquosorum* with a similarity index of 99.36% (Table 4).

Table 4. Molecular identification of selected antagonistic bacterial strains using 16S rRNA genes with the corresponding GenBank accession numbers.

Bacterial Strain	GenBank Closest Relative	Accession No	Per. Identity
B7	<i>Bacillus tequilensis</i> strain IKAK46	MZ314516.1	100
B21	<i>Bacillus subtilis</i> subsp. <i>Inaquosorum</i>	LN556353.1	99.36

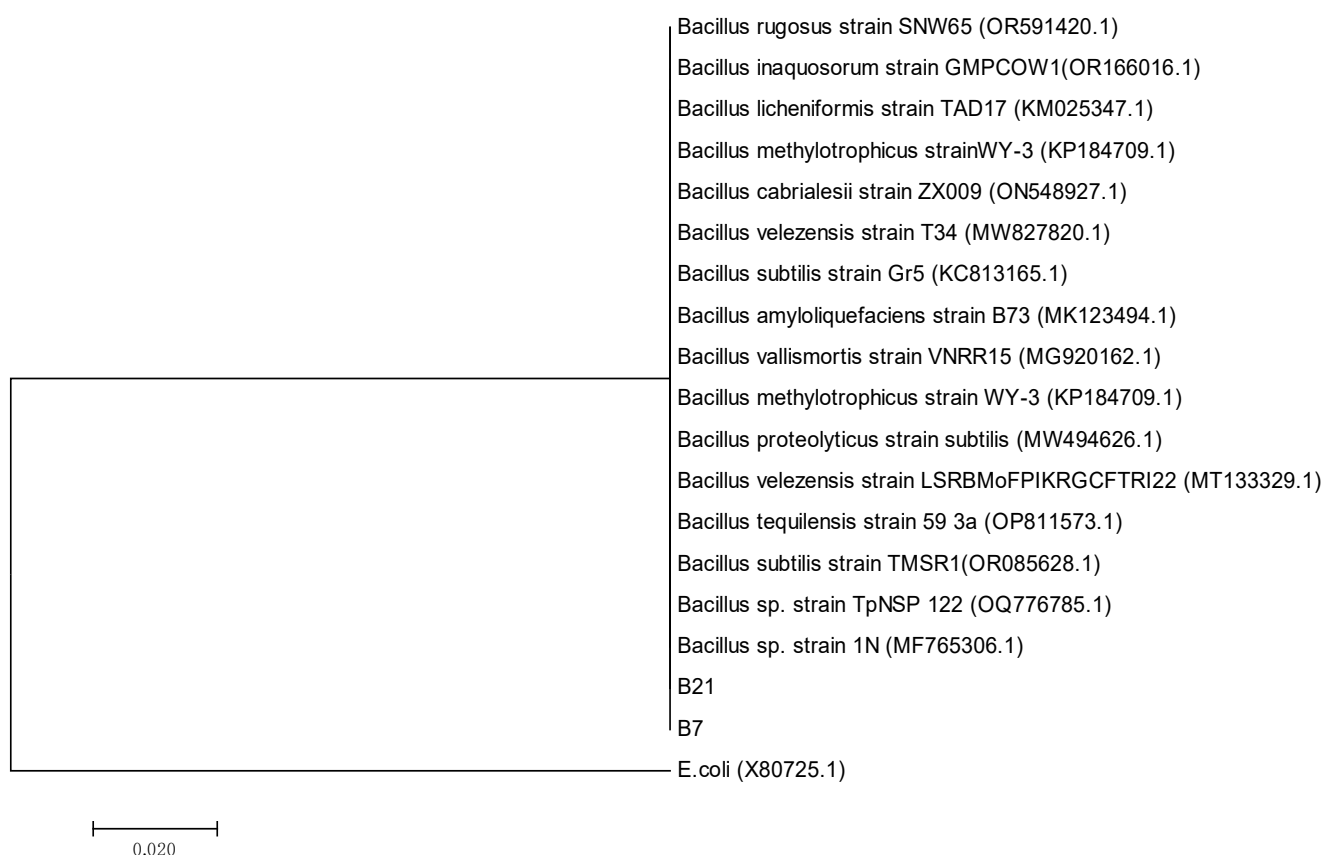


Figure 2. Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura-2 model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA7.

3.3. Confrontation Assay/Mycophagous Activity

Microscopic examination revealed the inhibitory effects of strain B7 on *F. solani* mycelial growth at 24, 48, and 72 h in the B7 + *F. solani* treatment (Table 5). At 24 h, a reduced number of hyphal branches was observed in the B7 + *F. solani* treatment compared to the *F. solani*-only control (FSF). By 48 h, there was a slight increase in hyphal branching in the B7 + *F. solani* treatment relative to 24 h, but this remained lower than the control. In contrast, the *F. solani* control showed a consistent increase in hyphal development throughout the incubation period.

Table 5. The number of branches of the mycelium of *F. solani* during the liquid confrontation assay between *F. solani* and *Bacillus tequilensis* strain IKAK46 B7.

Time (h)	Number of Branches per <i>F. solani</i> Hyphal Segment	
	F. S	B7 + F.S
24	9.00 ± 0.28 ^c	3.09 ± 0.28 ^e
48	10.32 ± 0.48 ^b	5.61 ± 0.13 ^d
72	34.61 ± 0.28 ^a	2.43 ± 0.13 ^e
C.V	8.04	
LSD.	1.57	
<i>p</i> >	0.0001	

The values indicate that the mean number of branches in 5 hyphals from three biological replicates ($n = 3$) with the same superscript letter(s) within the same column is not significantly different according to Fisher's test. According to Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d, e) indicate significantly different means within a column. Data are presented as mean ± standard error.

3.4. Screening for Antibiosis-Associated Genes

The primers employed targeted genes associated with the biosynthesis of antibiotic metabolites active against phytopathogens. Among the six genes screened, three were successfully amplified by PCR (Table 6): *phlD* (involved in the production of 2,4-diacetylphloroglucinol), *prnD* (responsible for pyrrolnitrin synthesis), and *hcnBC* (linked to hydrogen cyanide production). The *phzCD* gene, which encodes enzymes for phenazine-1-carboxylic acid synthesis, was not detected. All amplified PCR products matched the expected size (Table 2).

Table 6. In vitro screening for genes associated with antibiosis genes.

Bacterial Strain	Antibiosis Genes					
	<i>phlD</i> (2,4-DAPG)	<i>phzCD</i> (phenazine-1-carboxylic acid)	<i>prnD</i> (pyrrolnitrin)	<i>pltC</i> (pyoluteorin)	<i>pltB</i> (pyoluteorin)	<i>hcnBC</i> (hydrogen cyanide)
B7	+	—	+	—	—	+

Note: +: present; —: a3.5. Production of lytic enzyme activity.

3.4.1. Chitinase Activity

Exochitinase activity, measured in terms of GlcNAc monomer production, was highest in the *F. solani* (F.S) treatment, ranging from 18.0 to 32.3 U/mL, compared to the B7 + F.S (7.0–9.3 U/mL) and B7 (1.3–3.7 U/mL) treatments (Table 7). In contrast, chitinase activity based on GlcNAc-2 was lowest in the F.S. treatment but significantly higher in B7 + F.S and B7 treatments, with the peak activity (3160.0 U/mL) observed at 72 h. A time-dependent increase in chitinase activity was evident in the B7 + F.S treatment. Endochitinase activity (GlcNAc-3) was predominantly detected in B7 and B7 + F.S treatments. While a decrease was noted in B7 alone over time, the B7 + F.S treatment exhibited a steady increase from 24 to 72 h, indicating a synergistic enhancement of enzyme production during bacterial–fungal interaction.

Table 7. Chitinase activity between B7 and *F. solani*.

Time (h)	Chitinase Activity (U/mL)								
	Exochitinase						Endochitinase		
	GlcNAc			GlcNAc-2			GlcNAc-3		
	B7	F. S	B7 + F.S	B7	F. S	B7 + F.S	B7	F. S	B7 + F.S
24	3.67 ± 0.33 ^f	32.33 ± 0.88 ^a	7.00 ± 0.58 ^e	2544.00 ± 202.21 ^b	4.67 ± 0.88 ^c	2563.00 ± 55.64 ^b	1214.33 ± 41.95 ^a	22.67 ± 1.20 ^f	850.67 ± 12.72 ^d
48	1.67 ± 0.33 ^g	26.33 ± 1.20 ^b	7.67 ± 0.67 ^{de}	3084.00 ± 10.21 ^a	6.00 ± 1.16 ^c	2984.00 ± 51.23 ^a	959.00 ± 35.37 ^{cd}	24.00 ± 0.58 ^f	1038.33 ± 31.93 ^{bc}
72	1.33 ± 0.33 ^g	18.00 ± 0.58 ^c	9.33 ± 0.33 ^d	2625.67 ± 68.37 ^b	3.00 ± 0.58 ^c	3160.00 ± 5.12 ^a	709.00 ± 95.25 ^e	21.00 ± 0.58 ^f	1108.67 ± 6.12 ^{ab}
C.V		9.4			7.02			10.08	
LSD		1.93			227.2			114.24	
p>		0.0001			0.0001			0.0001	

The values indicate that the mean of three duplicates in duplicate ($n = 6$) with the same superscript letter(s) within the same column is not significantly different according to Fisher's test. According to Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d, e, f, g) indicate significantly different means within a column. *N*-acetyl- β -D-glucosaminide (GlcNAc); *N,N'*-diacetyl- β -D-chitobioside (GlcNAc-2) for exochitinase activity; and β -D-*N,N',N''*-triacetylchitotriose (GlcNAc-3) for endochitinase activity. Data are presented as mean $\pm$ standard error.

3.4.2. Chitosanase Activity

Chitosanase activity was significantly higher in the *B7 + F. solani* treatment compared to B7 alone, regardless of the incubation period. Across all treatments, chitosanase activity progressively increased from 24 to 72 h. The highest zone of inhibition was observed in the *B7 + F. solani* treatment at 72 h, measuring 29.0 ± 1.15 mm (Table 8). This indicates a synergistic induction of chitosanase production during the interaction between strain B7 and *F. solani*.

Table 8. Chitosanase activity between *F. solani* and B7.

Time (h)	Chitosanase Activity (mm)	
	B7	B7 + <i>F. solani</i>
24	10.62 ± 0.23 ^d	12.07 ± 0.51 ^{cd}
48	12.05 ± 0.18 ^d	28.33 ± 0.38 ^b
72	15.13 ± 0.28 ^c	26.10 ± 1.25 ^a
C.V	7.43	
LSD	2.41	
p>	0.0001	

The values indicate that the mean of three duplicates in duplicate ($n = 6$) with the same superscript letter(s) within the same column is not significantly different according to Fisher's test. According to Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d) indicate significantly different means within a column. Data are presented as mean $\pm$ standard error.

3.5. In Vivo Antagonistic Potential of Selected B7. Using Pot Assays

To evaluate the protective effect of *Bacillus* strain B7 against *Fusarium solani*, the percentage of disease incidence was assessed in both B7-treated and untreated common bean plants (Figure 3). Disease symptoms appeared in both groups by Day 7 post-inoculation. However, a marked difference in disease progression was observed. By Day 14, untreated plants exhibited a sharp increase in disease incidence, reaching 79.3%, whereas B7-treated plants showed significantly lower incidence at 35.6%. These results suggest that strain B7 provides substantial protection against *F. solani* infection, effectively reducing disease development over time (Figure 3).

Figure 4 presents data on the number of wilted common bean plants over a 21-day period, highlighting the effect of *Bacillus* strain B7 inoculation on plants challenged with *Fusarium solani*. The untreated group (inoculated with *F. solani* only) exhibited a progressive increase in the number of wilted plants from Week 1 to Week 3, with the highest incidence recorded in Week 3 (18.0 wilted plants). In contrast, plants treated with

strain B7 (B7 + *F. solani*) showed substantial protection, with markedly fewer wilted plants throughout the observation period. Within the treated group, no statistically significant differences were observed between Week 1 and Week 2, or between Week 2 and Week 3. However, a significant difference was observed between Week 1 and Week 3, with wilted plant counts slightly increasing from 2.0 (Week 1) to 5.0 (Week 3). These findings underscore the protective effect of strain B7 in mitigating disease severity caused by *F. solani* in common beans (Figure 4).

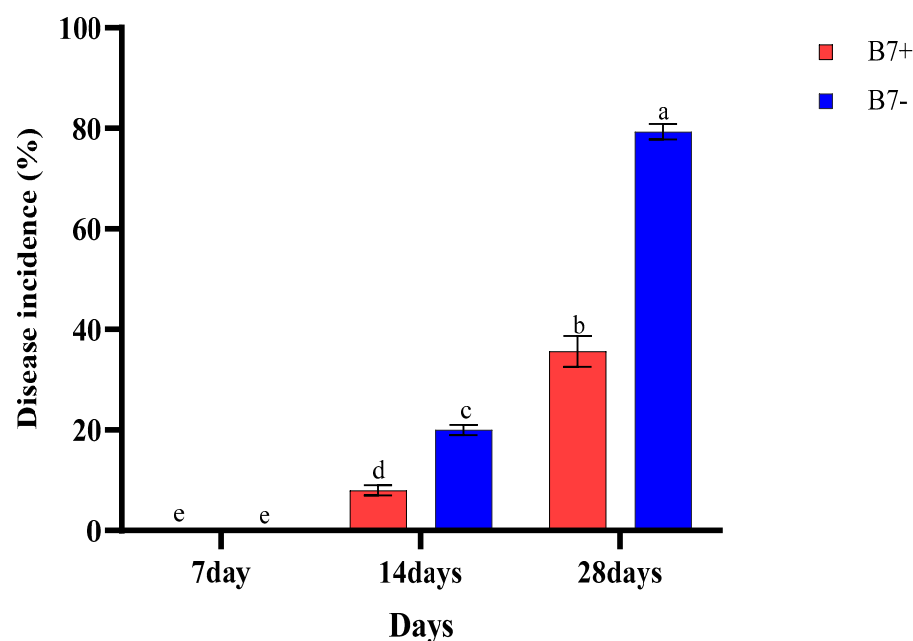


Figure 3. Percentage of disease incidence based on symptomatic plants per total number of plants observed infected with *F. solani* on the root of common beans, B7+: B7 treated; B7–: not treated. According to Fisher’s LSD test ($p < 0.05$), different superscript letters (a, b, c, d, e) indicate significantly different means within each parameter. The error bars indicate standard error (SE).

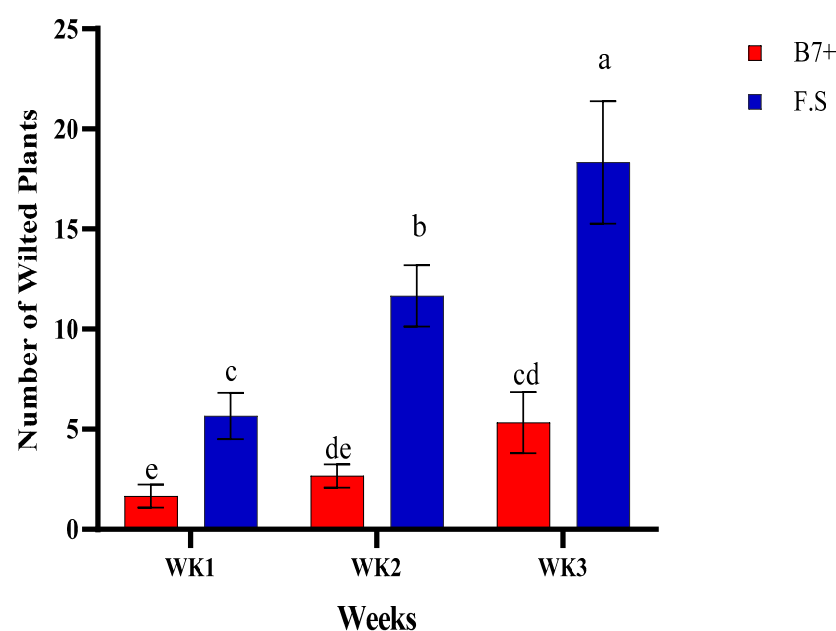


Figure 4. Number of wilted plants observed for 3 weeks, B7+: B7 bacterial strain treated; F.S: *Fusarium solani* treated. According to Fisher’s HSD test ($p < 0.05$), different superscript letters (a, b, c, d, e) indicate significantly different means within each parameter. The error bars indicate standard error (SE).

3.6. Survival and Population Density of the B7 Strain in Common Bean Roots

The population density of *Bacillus* strain B7 on the roots of common bean plants was assessed using the colony-forming unit (CFU) counting method (Figure 5). Fluorescent observations of treatments T1 (B7 + *F. solani*) and T3 (B7 only) revealed viable bacterial populations on the root surfaces. The CFU counts, expressed as Log_{10} values, ranged from 5.8 to 7.0 for T1 and from 5.8 to 8.4 for T3. In contrast, no detectable CFUs were observed in treatments T2 (*F. solani* only) and T4 (untreated control), both of which recorded a count of 0 CFU. Notably, CFU levels in T1 and T3 increased during the first 14 days post-inoculation, followed by a gradual decline over time, indicating successful initial colonization of the root system by strain B7 and subsequent stabilization of its population dynamics (Figure 5).

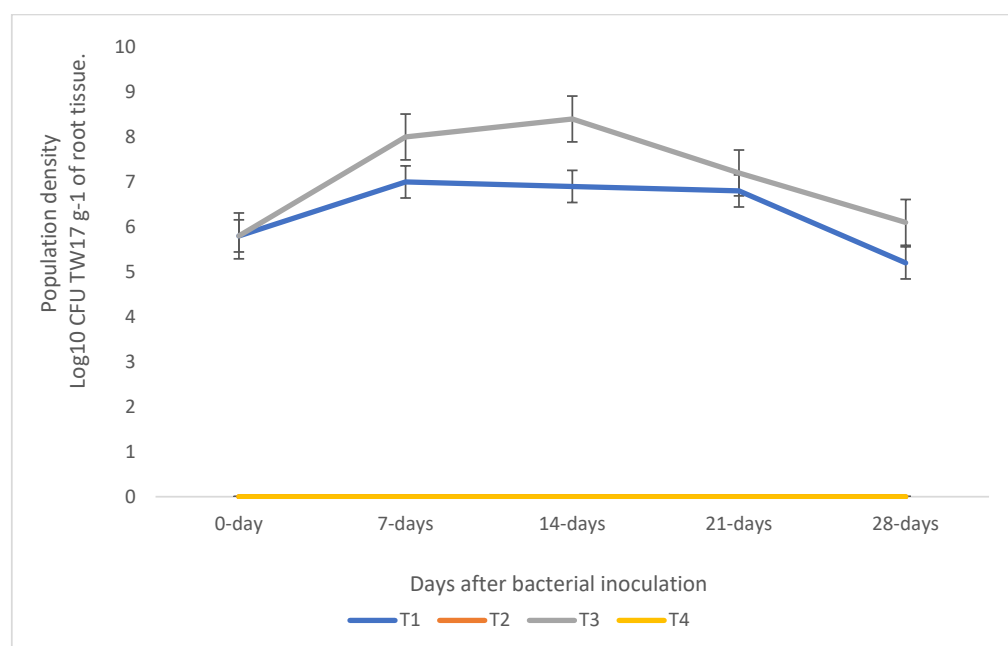


Figure 5. Population density of B7 strain on common bean root in greenhouse determined by the CFU counting method. Line T1: CFU of fluorescent B7 strain in treatment with B7 + *F. solani* treatment; T2: treatment with *F. solani*-only treatment; T3: CFU of B7 strain in treatment with B7-only treatment; T4: Absolute control (without pathogen or bacteria strain). The error bars indicate standard error.

4. Discussion

The potential of microorganisms from soda lakes as biocontrol agents against phytopathogens has been previously highlighted by Wekesa et al. [47]. Building on this concept, the current study investigated *Bacillus* spp. isolates from Lake Bogoria for their antagonistic activity against *Fusarium solani*, a major pathogen of common beans. Out of 30 bacterial isolates screened, 10 demonstrated varying degrees of mycelial growth inhibition. Notably, isolates B7 and B21 exhibited the highest antagonistic efficacy, each achieving inhibition rates exceeding 40%. These findings are consistent with those of Dugassa et al. [50], who reported that the cell-free culture filtrates of *Bacillus* spp. could suppress *Fusarium* mycelial growth by up to 71.11%. The observed inhibitory effects in our study may be attributed to the production of cell wall-degrading enzymes (CWDEs), as previously documented by Nehra et al. [28], who associated *Bacillus*-mediated antagonism with enzymatic lysis of fungal cell walls. Additionally, the inhibition may result from the secretion of diffusible antimicrobial compounds that interfere with fungal growth and proliferation [51].

The antagonistic interaction appeared to intensify between 48 and 72 h' post-contact, suggesting inducible enzymatic responses during direct confrontation. Similar results were

reported by Baard et al. [25], who described the enzymatic degradation of pathogenic fungal cell walls by biocontrol microbes in direct physical association. Thus, the mycelial inhibition observed in this study likely results from both antibiosis and enzyme-mediated suppression, reinforcing the biocontrol potential of *Bacillus* isolates from Lake Bogoria.

The results based on 16S rRNA; BLAST confirms the taxonomical identity of B21 as *Bacillus subtilis* subsp. *Inaquosorum*.

The isolate B7 demonstrated 97.5% sequence identity to known *Bacillus* species, suggesting it may represent a genetically distinct strain within the genus. Previous studies have documented the presence of *Bacillus* spp. and other Gammaproteobacteria in extreme environments, including soda lakes [52], with Gammaproteobacteria frequently identified as a dominant class. The sequence divergence observed in isolate B7 could reflect adaptive evolution to the harsh physicochemical conditions of Lake Bogoria, highlighting the potential of underexplored extreme environments as reservoirs for novel microbial strains with agronomic applications.

Following initial screening, isolate B7 was subjected to further molecular characterization to investigate the presence of genes associated with antibiosis. PCR amplification revealed the presence of several biosynthetic genes, including *hcn*, *plt*, *prn*, *phz*, and *phl*, which are involved in the production of hydrogen cyanide, pyoluteorin, pyrrolnitrin, phenazine, and 2,4-diacetylphloroglucinol (2,4-DAPG), respectively [53,54]. Notably, previous work by Kumar et al. [55] has reported the production of pyoluteorin by *Bacillus* spp., supporting our findings.

We also evaluated the production of lytic enzymes—specifically chitinase and chitosanase—which are key in fungal cell wall degradation [56]. Enzymatic assays revealed both exochitinase and endochitinase activity at 24-, 48-, and 72-h post-confrontation in the treatment with B7 and *F. solani*. Endochitinase cleaves internal bonds in the chitin polymer, creating substrates for exochitinase to further degrade the fungal cell wall. As chitin and its derivative chitosan are major structural components of the cell walls in filamentous fungi [57], degradation by bacterial chitosanase—alongside chitinase—plays a critical role in hyphal lysis [58]. The enzymatic action likely contributed to the observed reduction in hyphal branching and morphological abnormalities in *F. solani*, as visualized under light microscopy. Similar antagonistic effects have been reported by Báez-Astorga et al. [41] in *Bacillus* interactions with *Rhizoctonia solani*.

In the in vivo experiment, root colonization by strain B7 was confirmed using a culture-based approach. A higher recovery of fluorescent *Bacillus* colonies was recorded from roots treated with B7 compared to untreated controls, indicating successful root colonization even in the presence of *F. solani*. This observation is consistent with the findings of Bakki et al. [59], who emphasized the importance of microbial persistence for effective disease suppression. Colony counts from root samples confirmed that B7 maintained viable populations above the efficacy threshold required for biocontrol activity.

Moreover, disease incidence assessments revealed that plants treated with B7 exhibited significantly lower rates of wilting compared to those inoculated with *F. solani* alone. This protective effect is likely mediated through both direct antagonism and the induction of host defense responses, a well-recognized mode of action in plant-associated biocontrol bacteria [59].

In conclusion, *Bacillus* spp. isolated from Lake Bogoria exhibited potent antagonistic activity against *F. solani*, both in vitro and in vivo. Strain B7, in particular, demonstrated multiple biocontrol mechanisms, including production of antifungal secondary metabolites, lytic enzymes (chitinase and chitosanase), and robust root colonization. These attributes, combined with its adaptation to extreme environmental conditions, position B7 as a promis-

ing candidate for biocontrol applications, especially in arid and semi-arid lands (ASALs) where traditional biocontrol agents often fail to perform effectively.

Despite the promising results, this study is limited by the absence of field trials, which are essential to validate B7's efficacy under natural agronomic conditions. Additionally, whole-genome sequencing of strain B7 was not conducted, which would be critical for confirming its novelty and biosynthetic potential. Nevertheless, the study's strengths lie in its integrative approach—combining environmental microbiology, gene profiling, enzymatic assays, and in vivo evaluation—culminating in the identification of a highly promising biocontrol strain.

The findings support the broader potential of extremophilic microorganisms from soda lakes as sustainable alternatives to chemical fungicides. Strain B7, with its demonstrated antifungal activity, root colonization capacity, and environmental resilience, offers a valuable opportunity for advancing climate-smart agriculture. Future research should prioritize field validation and genomic analysis to fully harness the potential of such extremophile-derived biocontrol agents for use in Kenyan agriculture and beyond.

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