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RESEARCH PAPER

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Taxonomical, functional, and cytopathological characterization of *Bacillus* spp. from Lake Magadi, Kenya, against *Rhizoctonia solani* Kühn in *Phaseolus vulgaris* L

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Abstract

A decline in common bean production and the ineffectiveness of synthetic chemical products in managing plant pathogens has led to exploiting Kenyan soda lakes as an alternative search for biocontrol agents. This study aimed to identify phylogenetically *Bacillus* spp. from Lake Magadi and their antagonistic activity against *Rhizoctonia solani* under in vitro and in vivo conditions. The 16 S ribosomal RNA (rRNA) subunit sequences of six bacterial strains isolated from Lake Magadi showed diversity similar to the *Bacillus* genus; *Bacillus velezensis*, *Bacillus subtilis*, and *Bacillus pumilus*. In vitro, antagonism showed varied mycelium inhibition rates of fungi in the coculture method. Enzymatic assays showed the varied ability of isolates to produce phosphatase, pectinase, chitinase, protease, indole-3-acetic acid (IAA), and hydrogen cyanide (HCD). The in vivo assay showed M09 (*B. velezensis*) with the lowest root mortality and incidence of postemergence wilt. Pre-emergence wilt incidence was recorded as lowest in M10 (*B. subtilis*). Isolate M10 had the highest phenylalanine ammonia-lyase (PAL) for defense enzymes, while polyphenol oxidase (PPO) and peroxidase were recorded as highest in M09. For the phenolic content, M10 recorded the highest phenolic content. In conclusion, Lake Magadi harbors *Bacillus* spp, which can be used as a potential biocontrol of *R. solani*.

KEYWORDS

Bacillus spp, biocontrol, characterization, Lake Magadi, *Rhizoctonia solani* Kühn

1 | INTRODUCTION

Common beans (*Phaseolus vulgaris L.*) are an essential cash crop and a source of protein, especially for low-income families. It is mainly grown for subsistence and is valued at USD 452 million [1]. It contains protein and calories and is recognized as "poor man's meat" due to its low price compared with other sources of proteins [2, 3]. Beans contain significant minerals such as zinc, iron, and vitamins [2, 4]. It also has essential nutrients such as ascorbic acid, vitamins A and B, and calcium. Bean production is mainly affected by biotic and abiotic

Abbreviations: BLAST, Basic Local Alignment Search Tool; CFU, colony forming units; HCD, hydrogen cyanide; IAA, indole-3-acetic acid; ISR, induction of systematic resistance; KIRDI, Kenya Industrial Research and Development Institute; KWS, Kenya Wildlife Service; LSD, least significant difference; MEGA, Molecular Evolutionary Genetics Analysis; NCBI, National Center for Biotechnology Information; PAL, phenylalanine ammonia-lyase; PCR, polymerase chain reaction; PO, peroxidase; PPO, polyphenol oxidase; UON, University of Nairobi..

factors. Root rots have been frequently reported as a major threat to production in Kenya [5, 6]. It is caused by pathogens such as bacteria, fungi, and nematodes which are causative agents. Some of the fungi such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Aspergillus, Colletotrichum* and *Aspergillus* spp. severely affects the crop among Kenyan farmers [7]. According to Al-Hazmi and Al-Nadary [8], *R. solani* is a common pathogen most devastating in both large and small-scale farmers. It contributes to >80% loss in yield worldwide [9]. The pathogen is found in soil, air, and moist environment; therefore, difficult to manage through conventional methods. It can also endure hyphae in disease crop debris and survive in the soil for an extended period [10]. Additionally, it is linked to causing damping off diseases, reddish-brown canker, and yellowing of a plant.

A different approach, such as traditional practices and chemical methods, has been employed in managing *R. solani*. These methods have not been effective, cause environmental degradation, and affect nontarget beneficial microorganisms [11]. Additionally, synthetic fungicides are linked to increased resistance to target microorganisms due to continuous exposure to chemical-fungicides hence, causes genetic mutation. Biocontrol agents are possible alternative strategies to chemical methods due to their importance, such as being more sustainable and environmentally friendly than chemical pesticides [5]. They can be used in a targeted way, reducing the risk of nontarget effects and minimizing the accumulation of toxins in the environment. Second, it is cost-effective since it requires less input and maintenance and can help prevent crop losses. Lastly, resistance management provides an alternative mode of action [12]. Biocontrol agents employ different mechanisms, such as predation, parasitism, competition, and induced resistance. Due to their wide mode of action, they are used in managing predatory insects, parasitic wasps, fungal and bacterial diseases, and nematodes.

In agriculture, *Bacillus* spp. is reported as a biocontrol agent and shows the potential to control plant pathogens [13]. For instance, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus mycoides*, and *Bacillus thuringiensis* are mostly exploited as biocontrol. They are of great agricultural importance [14] since, able to suppress the growth of several fungal mycelia such as *Rhizoctonia*, *Fusarium*, *Gaeummanomyces*, *Nectria*, *Pythium*, *Phytophthora*, and *Verticillium* [15–18]. The ability to suppress pathogens is due to different mechanisms. Among the proposed mechanism is the production of lytic enzymes capable of degrading pathogen cell walls. For instance, Wekesa et al. [13] reported *Bacillus velezensis* suppressing the mycelium growth of *Fusarium solani*, causing fusarium wilt incidence by producing secondary metabolites and through competition. Marach et al. [19] also reported using *B. amyloliquefaciens* to biocontrol bacterial leaf blight on rice seedlings by producing secondary metabolites such as chitinase.

Moreover, *Bacillus* spp. Produces a range of other metabolites, such as chitinases, phosphatase, pectinase, Indole-3-acetic acid (IAA), hydrogen cyanide (HCN), and proteases, as mechanisms to inhibit the growth of pathogen and improve plant growth [15, 20]. They can inhibit fungal mycelium's growth by degrading the cell wall, producing antibiosis enzymes, competition, mycoparasitism, and induced resistance. This study investigated *Bacillus* spp. as a biocontrol agent against *R. solani*. For this purpose, the *Bacillus* spp. were isolated from the soil and sediment of Lake Magadi, screened for antifungal and enzymatic activity, and identified through molecular technique. Greenhouse assays were used to confirm the efficacy of the selected *Bacillus* spp. and its ability to produce defense enzymes such as phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), peroxidase (PO), and total phenolic content.

2 | MATERIALS AND METHODS

2.1 | Isolation of bacteria

The isolation n of *Bacillus* spp. from soil and sediment was carried out using methods described by Wekesa et al. [13]. In brief, 1 g of soil and sediment were weighed and then homogenized in a sterile test tube containing 9 mL of sterile physiological saline (0.85% NaCl). The resulting suspensions were then vigorously vortexed at 150 rpm for 1 min. A fivefold serial dilution of the suspensions was done in a ratio of 1:9 up to 10^{-4}. An aliquot of 30 µL from dilutions 10^{-3} and 10^{-4} was cultured according to Hartman [21] on modified nutrient agar-Himedia as described by Wekesa et al. [13]. The plates were incubated at 35°C for 48 h. Based on colony morphology, distinct colonies were isolated and purified separately on an isolation medium. The pure bacterial isolates were characterized using standard microbiological techniques as described by Wekesa et al. [13] and cell morphology was done by Gram staining technique as described by Tripathi and Sapra [20] using a light microscope (MD827S30L).

2.2 | Screening of *Bacillus* spp. for antagonistic activity using coculture plate assays

The antagonistic activity of *Bacillus* spp. was evaluated against *R. solani* using the coculture technique described

by Aydi et al. [22]. In this assay, fungi and bacteria were cultured on potato dextrose agar-Himedia. The plugs were placed at the end of the petri dish, and bacteria (10^8 cfu/mL) were streaked perpendicularly across the Petri dish. In contrast, control plates were inoculated with only the pathogen at the center of the Petri-dish. The plates were incubated at 30°C for 14 days. The percentage inhibition rate (I.R.) of *R. solani* mycelium was calculated using the formula described by Aydi et al. [22].

Formula

$$\%I.\,R. = \frac{C2 - C1}{C2} \times 100, \quad (1)$$

where I.R is the inhibition rate, C2 the colony diameter of the pathogen in control, and C1 the colony diameter of the pathogen cocultured with bacteria.

2.3 | Molecular identification of selected antagonistic bacterial isolates

The selected antagonistic bacterial isolates were identified using 16 S ribosomal RNA (rRNA) gene amplification and sequencing. The genomic DNA extraction was performed using a bacterial DNA isolation kit (Norgen Biotek Corp.) according to the manufacturer's instructions. Bacterial DNA was then quantified using Qubit (Qubit 4 Fluorometric quantification, Q33238), and its concentrations were adjusted to 100 ng of DNA/μL.

The amplification was done using universal bacterial primers 27 F (5'-AGAGTTTGATCCTGGCTCAG.−3') and 1492. R (5'-CGGCTACCTTGTTACGACTT-3'). Polymerase chain reaction (PCR) amplification was performed using Peqlab Primus 96 thermocycler (PEQLAB). The amplification was done in a 40 μL mixture comprising 20 μL of master mix, 18.2 μL of PCR water, 0.4 μL of the 27 F primer, 0.4 μL of the 1492 R primer, and 1 μL of template DNA (10 ng/mL).

The temperature cycling profiles were applied for the reaction mixtures described by Wekesa et al. [13]. The presence and size of the PCR amplicon were verified on 1.2% agarose gel and visualized under UV light [23]. The PCR amplicons were purified using the QIAquick PCR amplification kit protocol (Qiagen) according to the manufacturer's instructions. The PCR amplicon was sent to Macrogen for sequencing.

The homology search was performed using Basic Local Alignment Search Tool (BLAST) algorithm from the National Center for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences with the greatest similarity from the BLAST findings were selected and aligned using CLUSTAL W 2.0. cooperated in MEGA 7 for pairwise and multiple sequence alignment, where the Kimura-2 parameter model was used to calculate the evolutionary distances and build the phylogeny tree.

2.4 | Functional characterization of selected bacterial isolates

2.4.1 | Protease activity

The proteolytic activity of bacterial isolates was assessed as Saran et al. described [24]. In brief, bacterial isolates were cultured on skim milk agar-Himedia (28.0 g S.M. powder, 5.0 g of tryptone, 2.5 g of yeast extract, 1.0 g of dextrose or glucose, and 15.0 g of agar) per liter. The plates were incubated at 30°C for 24 h. The diameter of the observed clear zone around the colony was measured and recorded in millimeters.

2.4.2 | Chitinase activity

The chitinolytic activity of bacterial isolates was screened as described by Banerjee et al. [25]. The bacterial isolates were inoculated into colloidal chitin agar medium (Na_2HPO_4, 6 g/L; KH_2PO_4, 3 g/L; NH_4Cl, 1 g/L; NaCl, 0.5 g/L; yeast extract, 0.05 g/L, agar, 15 g/L, and colloidal chitin 1% (w/v)) and incubated at 30°C for 5 days. The colony diameter and clear zone diameter were calculated using the Chitinolytic Index formula [26].

$$CI = \frac{\text{clear zone diameter} - \text{colony diameter}}{\text{colony diameter}} \quad (2)$$

where CI = Chitinolytic Index.

2.4.3 | Pectinase activity

Pectinase production was assessed by inoculating bacterial suspensions (~ 10^8 cells/mL) on the nutrient agar-Himedia supplemented by pectin (5 g/L) (w/v). Plates were incubated at 30°C for 48 h, and the presence or absence of a clear zone around the colonies was assessed.

2.4.4 | Production capacity of HCN

HCN was qualitatively detected, as described by Rijavec and Lapanje [27]. The bacterial isolates were

inoculated on nutrient agar-Himedia supplemented with glycine (4.4 g/L) (w/v). Sterile discs (9 mm diameter) soaked in picric alkaline solution (2% sodium carbonate and 0.5% picric acid) were placed on the lid of each Petri dish. Standard HCD was used as a control for comparison. Plates were sealed with parafilm and incubated at 25°C for 4 days. The color change from yellow to light-reddish brown indicates positive HCN production.

2.4.5 | Phosphate solubilization capacity

According to Tamrela et al. [26], bacterial isolates' ability to solubilize phosphate was done qualitatively. Briefly, bacterial isolates were cultured on the Pikovskaya-Himedia, whereas sterile water was used as a control. The cultures were incubated at 30°C for 7 days. A clear zone formed around the colonies was measured and recorded.

2.4.6 | Production capacity of IAA

IAA was detected, according to Gang et al. [28]. Luria-Bertani broth-Himedia (LB) was prepared with exogenous tryptophan (4 mg /mL). Bacterial cells (~10^8 CFU/mL) were inoculated into 10 mL LB, supplemented with tryptophan in 50 mL flasks. Samples were incubated for 7 days under dark conditions on a shaker incubator at 120 rpm. Bacterial cultures were centrifuged at 12,000 rpm for 10 min at 4°C. After centrifugation, 1 mL supernatant from each sample was mixed with 2 mL Salkowski reagent (98 mL 35% $HClO_4$, 2 mL 0.5 M $FeCl_3$) and incubated in the dark for 30 min. The change of color from yellow to pink was considered positive for IAA production.

2.5 | In vivo antagonistic potential of selected *Bacillus* spp. using pot assays

The antagonistic effects of M09 (*B. subtilis*) and M10 (*B. velezensis*) against *R. solani* were assayed under greenhouse conditions using the pot culture method as described by Baazeem et al. [16]. The seeds were surface sterilized with 70% ethanol for 30 s and 5% NaOCl for 5 min and rinsed three times with sterile distilled water. Soil preparation (manure: soil: sand in a ratio of 1:3:2 (v/v)) as the plant growth medium was carried out by steam sterilization and left to cool before being placed in sterilized pots. Pathogen inoculums containing 2×10^8 spores/mL were mixed into the potting medium at 150 mL/kg of soil and incubated for 5–7 days to achieve proper spore germination and establishment of the pathogen mycelium. The surface-sterilized seeds were sown in each pot (five seed pot^{-1}), and daily observations were taken for wilt incidence. The pot bioassay treatment under greenhouse conditions included P1—absolute control (without pathogen and bacteria); P2—*R. solani* only (positive control); P3—M09 + *R. solani*; P4—M10 + *R. solani* treatments. The experimental design consisted of five replications for each treatment.

2.5.1 | Determination of plant biometric attribute

Root mortality

Pathogen-induced mortality in plant roots was assessed in the presence or absence of bacterial agents. Mortality caused in seedling roots due to soil inoculation with *R. solani* was determined using a modified method described by Dukare and Paul [29]. Root mortality was expressed as the percentage of dead roots' dry weight against the roots' total dry weight.

Pre-emergence and postemergence wilt incidence

The pre- and postemergence wilt disease severity indices were calculated by counting the number of germinating and surviving seedlings described by Gossen et al. [30]. A percentage of disease incidences was calculated based on visible wilt symptoms observed in the plant after 15 days of inoculation.

Shoot/root length and biomass

The effects of bacteria agents on plant elongation parameters were assessed by measuring the shoot and root length (cm). In addition, plant biomass, such as fresh shoots and root weight (g), were determined after 35 days of inoculation.

2.5.2 | Plant defense enzymes and phenolic

Preparation of enzyme extracts

From each treatment, 1 g of 4-week-old leaf samples were homogenized in 1.5 mL of 50 mM Tris HCl buffer (pH 7.5) at 4°C in liquid nitrogen and centrifuged at 18,000 rpm for 20 min at 4°C. The resulting supernatant was collected in sterilized 2 mL Eppendorf tubes and stored in a deep freezer (−20°C) for further use as a crude enzyme extract. This enzyme extract was used for the PAL, PPO, and PO assay.

PAL, PPO, and PO assays

PAL activity was estimated based on the production of trans-cinnamic acid [31]. Enzyme activity was expressed as microgram cinnamic acid h^{-1} g^{-1} fresh plant weight. PPO activity was tested by measuring the change in the intensity of the color of catechol oxidation products [29]. The activity of the PPO enzyme was expressed as a change in absorbance at 495 nm per min^{-1} per g^{-1} of fresh plant weight. For the PO assay, 0.5 mL of crude enzyme extract was taken in a cuvette, and subsequently, 0.5 mL of 1% guaiacol solution and 1.5 mL of 50 mM Tris buffer (pH 7.5) were added. The reaction was then started by adding 0.5 mL of 1% H_2O_2, and the change in absorbance at 470 nm was recorded at an interval of 30 s for 3 min. A unit of PO enzyme activity was expressed as the change in absorbance min^{-1} per g^{-1} of fresh weight.

Total phenolic assay

The total phenolic content in fresh leaf and root tissue was analyzed using the Folin–Ciocalteu colorimetric method [29]. The optical density of the developed blue color was measured at 725 nm. The phenolic content in the plant tissue was expressed as μg $catechol^{-1}$ g^{-1} fresh plant weight.

2.6 | Data analysis

Antifungal activity enzymatic assay and plant biomass were subjected to a one-way analysis of variance (ANOVA) using SAS version 8.0 software. For greenhouse data, GraphPad-Prism version 6.0 were used to present the data in graph format.

3 | RESULTS

3.1 | Isolation of bacteria

A total of 61 bacteria isolates were isolated from soil and sediment samples. Fifteen isolates had similar morphological characteristics to the genus *Bacillus* described by Logan and de Vos [32] regarding opacity, white colonies, velvety texture, large size, and irregular edges.

3.2 | Screening of *Bacillus* spp. for antagonistic activity using coculture plate assays

The selected *Bacillus* spp. inhibited the mycelium growth of *R. solani* at a range of 5.95%–42.86% in the coculture assay (Table 1). The plates were kept in an incubator for additional 7 days, and it was observed that the inhibition rate was persistent. Further, isolate M10, M9, and M47 showed good mycelium inhibition rate, as shown in Figure 1.

TABLE 1 Percentage mycelium inhibition rate of *Rhizoctonia solani* by selected bacterial strains.

Isolate code	Mycelium length (cm)	% Inhibition rate
Control	8.40 ± 0.00^{a}	0.00
M24	7.90 ± 0.46^{a}	5.95
M33	6.90 ± 0.10^{b}	17.86
M43	6.80 ± 0.30^{b}	19.05
M21	6.73 ± 0.35^{bc}	19.84
M32	6.63 ± 0.29^{bc}	21.03
M48	6.60 ± 0.26^{bcd}	21.43
M37	6.50 ± 0.4^{bcd}	22.62
M52	6.50 ± 0.30^{bcd}	22.62
M11	6.47 ± 0.45^{bcd}	23.02
M60	6.23 ± 0.2^{cde}	25.79
M50	6.10 ± 0.26^{de}	27.38
M16	5.87 ± 0.32^{e}	30.16
M47	5.30 ± 0.10^{f}	36.90
M09	5.03 ± 0.25^{f}	40.08
M10	4.80 ± 0.10^{f}	42.86

Note: The mean values with the same superscript letter(s) within the same column are not significantly different by the Fisher's test. According to the Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d, e, f) indicate significantly different means within a column.

3.3 | Molecular identification of selected antagonistic bacterial isolates

Sequencing was performed on only six isolates with the highest mycelium inhibition rate. From the partial sequences, the BLAST analysis showed that six isolates (100.00%) with high similarity (99.39%–100.00%) belonged to the *Bacillus* genus within the Firmicutes bacteria domain (Table 2). Among the *Bacillus* strains identified were *B. velezensis, B. subtilis*, and *B. pumilus* (Figure 2). Phylogenetic analysis of six isolates based on 16 S rRNA sequences allowed the isolates to be classified into three groups. The largest group (I) comprises four bacterial isolates (M50, M47, M16, and M09) affiliated with *B. subtilis*, with a high similarity of >99.39%. Group II, isolate M10, affiliated to *B. velezensis* with a 100.00% similarity identity. Group III isolated M60 affiliated with *B. pumilus* with a percentage identity of 100.00%

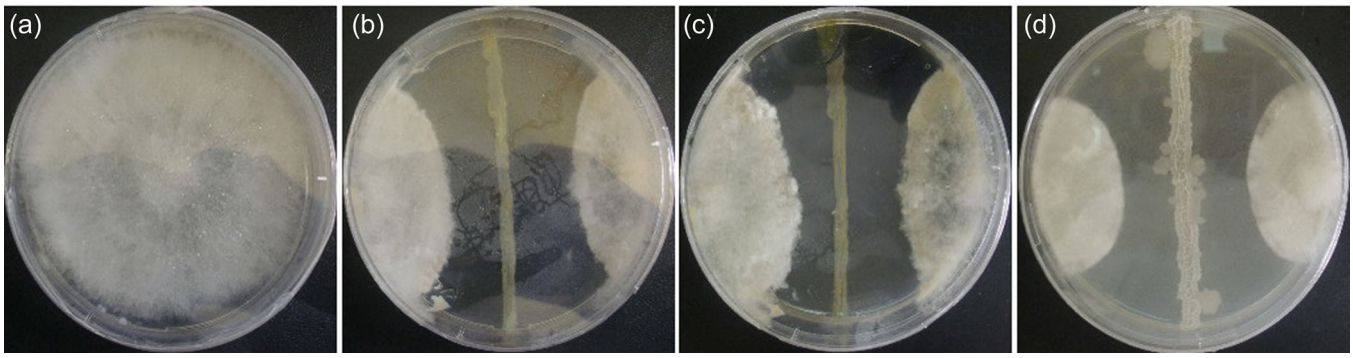

FIGURE 1 Antagonistic activity of bacterial strains. (a) control, (b) M10, (c) M09, and (d) M47 against *Rhizoctonia solani* using coculturing assays.

TABLE 2 Molecular identification of selected antagonistic bacterial strains using 16 S rRNA genes.

Isolate code	Acc. no.	Next neighbor in Blast	Sequence similarity (%)
M9	MT538513.1	*Bacillus subtilis* strain 3645	100.00
M10	MT012197.1	*Bacillus velezensis* strain CLT81	100.00
M16	KY206830.1	*Bacillus subtilis* strain Q235	99.93
M47	MT538489.1	*Bacillus subtilis* strain 3617	99.39
M50	MN966875.1	*Bacillus* sp. (in: Bacteria) strain S	100.00
M60	EU379282.1	*Bacillus pumilus* strain 4RS-5b 16 S	100.00

(Table 2). The group analysis showed the sequence diversity among *Bacillus* spp. that can be used as biocontrol.

3.4 | Enzymatic production bioassay

All the six selected antagonistic bacterial isolates were positive for protease activity, showing a clear zone around the colonies on skim milk agar plates, which indicates extracellular protease activity. The highest activity was recorded in M09 (Table 3, $p \leq 0.05$). For pectinase activity, none of the selected bacterial isolates showed a clear halo around the colonies on pectin agar (Figure 3). However, isolate M10, M47, and M50 produced phosphatase enzymes, with isolate M10 recording the highest activity (Table 3, $p \leq 0.05$). Strains M09, M47, and M50 exhibited a clear halo corresponding to chitinase on chitin agar. However, isolate M10 (1.53 ± 0.15 cm) exhibited the highest chitinase activity (Table 3, $p \leq 0.05$). None of the selected bacterial isolates produced sufficient HCD to be detected by picrate-impregnated filter paper (Figure 3). For IAA, only isolates M09, M10, and M60 tested positive.

3.5 | In vivo antagonistic potential of selected *Bacillus* spp. using pot assays

In the pot assay, the bacterial isolates M09 and M10 were used as the antagonistic isolates against *R. solani* in common bean plants. The isolates controlled the diseases caused by *R. solani* in bean plantlets, as shown in Figure 4. The diseases caused by *R. solani* were assessed by root mortality rate, pre and postemergence wilt incidence, length, and biomass. The root mortality rate was significantly reduced in treatments of the antagonistic bacterial isolates compared with the control (Figure 5).

The "positive control" received only fungal application. The highly significant ($p < 0.05$) treatments with antagonistic bacterial isolates observed pre- and post-emergence wilt incidence. The percentage of pre-emergence wilt incidence of *R. solani* with antagonistic strains was 18.00% and 22.00% compared with their positive control of 38.00% (Figure 6). The lowest postemergence was recorded in M09 and M10, with 32.00% and 39.00%, respectively, compared with positive control with 58.00% (Figure 6).

Compared with controls, the treatments with antagonistic bacterial isolates recorded highly significant (Table 4, $p \leq 0.05$) shoot and root length. The highest

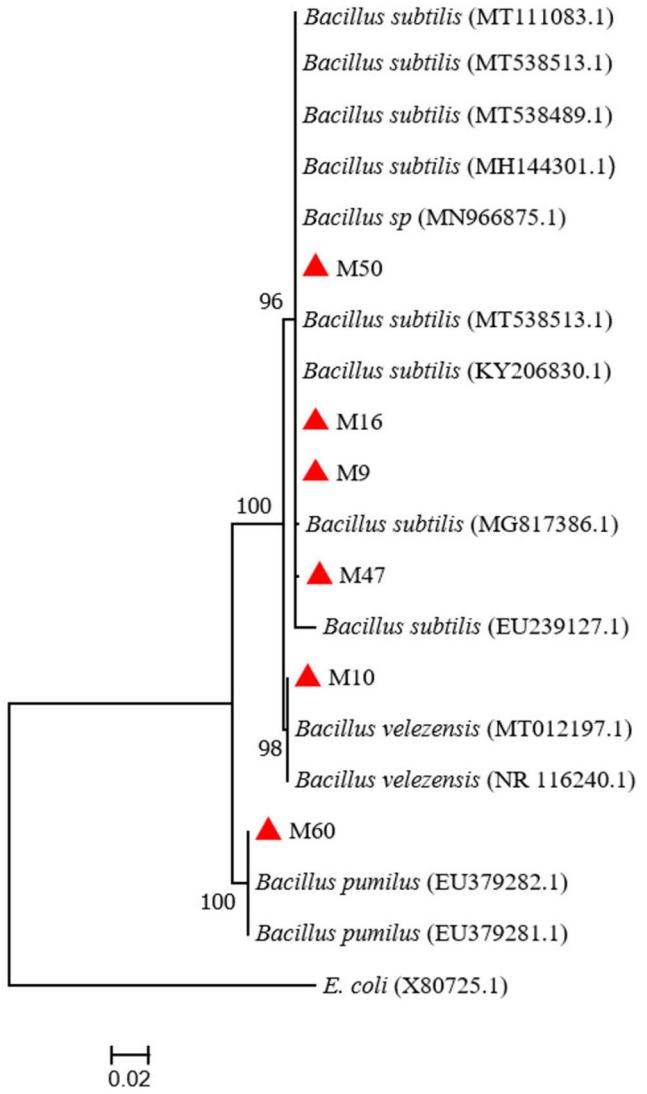

FIGURE 2 The evolutionary history of tested bacteria using the maximum likelihood method based on the Kimura-2 parameter model.

root and shoot length of 16.23 ± 0.72 cm and 16.83 ± 0.53 cm was recorded in the treatment P4. A maximum root weight (5.21 ± 0.23 g) and shoot weight (19.23 ± 0.35 g) were obtained in treatments P4 and P3, respectively. Hence, the bacterial isolates M09 and M10 controlled *R. solani* (Table 4).

3.6 | Production of secondary defense metabolites

In the presence of the pathogen, the highest level (321.04 ± 14.98) of PAL activity was recorded in the plant tissue of treatment P3, followed by treatment P4 (301.09 ± 13.82). The highest absorbance (0.08 ± 0.00) for PPO enzymes was obtained from treatment P4 (Table 5). However, PO build-up was enhanced by the presence of bacteria isolates. For instance, the highest PO (0.14 ± 0.01) was produced in the P3 compared with their positive controls (Table 5).

3.7 | Total phenolic content

The total phenolic content increased significantly (Figure 7, $p \leq 0.05$) in the leaf and root tissues of the antagonistic bacterial isolates compared with their controls. The highest phenolic content of 137.0 ± 5.8 μg catechol^{-1}g of fresh leaves wt. and 95.0 ± 5.2 μg catechol^{-1} g of fresh root wt. was observed in treatment P4 followed by treatment P3 with 133.0 ± 5.6 μg catechol^{-1} g of fresh leaves wt. and 93.0 ± 4.7 μg catechol^{-1} g of fresh root wt. (Figure 7). The lowest phenolic content was recorded in treatment P2 with 98.0 ± 5.0 μg catechol^{-1} g of fresh leaf weight and 80.0 ± 3.1 μg catechol^{-1} g of fresh root wt.

4 | DISCUSSION

This study explored *Bacillus* spp. from Lake Magadi as a potential biocontrol agent against *R. solani* in common beans. In our results, on the coculturing assay, 15 isolates of 61 that had similar characteristics of *Bacillus* spp. were screened for the ability to control the mycelial growth of *R. solani*. It was found that all of the isolates showed antagonistic ability and varied mycelium inhibition rate of the phytopathogen tested. Among them, isolates M09 and M10 had the highest antagonistic activity. Our results agree with Abbas et al.[11], who reported *Bacillus* spp. as the best biocontrol agent against *R. solani*. The mycelium inhibition is due to the synthesis of lytic enzymes produced by bacteria involved in cell degradation during antagonism [16, 33]. Furthermore, the mycelial inhibition rate may be due to diffusible bacterial inhibitory antibiosis substances, which could have suppressed and restricted pathogen growth [5].

The result based on 16 S rRNA, BLAST confirmed the taxonomical groups of the *Bacillus* strain isolates. All the isolates were of the genus *Bacillus* within the Firmicutes domains. The isolates identified in our study include *B. subtilis*, *B. velezensis*, and *B. pumilus*. These results agree with previous studies on Lake Magadi, where Firmicutes are the dominant bacteria domains [34]. Additionally, *Bacillus* spp. is one of the most common strains in aerobic, eubacterial alkaliphiles in soda lakes and normal environments.

From the study, these strains produce different enzymes, such as pectinase, phosphatase, chitinase, and

TABLE 3 Enzymatic bioassay of selected *Bacillus* spp.

Isolate code	Protease	Pectinase	Phosphatase	Chitinase	HCD	Indole-3-acetic acid
Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	+ve	-ve
M09	2.80 ± 0.06^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{d}	0.90 ± 0.06^{c}	-ve	+ve
M10	2.07 ± 0.03^{c}	0.00 ± 0.00^{a}	1.47 ± 0.03^{a}	1.53 ± 0.15^{a}	-ve	+ve
M16	2.07 ± 0.03^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	-ve	-ve
M47	1.47 ± 0.03^{d}	0.00 ± 0.00^{a}	1.20 ± 0.06^{c}	1.10 ± 0.06^{bc}	-ve	-ve
M50	2.30 ± 0.06^{b}	0.00 ± 0.00^{a}	1.33 ± 0.03^{b}	1.13 ± 0.09^{b}	-ve	-ve
M60	1.53 ± 0.03^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	-ve	+ve
CV	3.95	.	8.54	18.51		
LSD	0.12	0.00	0.09	0.22		
P>	0.0001	.	0.0001	0.0001		

Note: The mean values with the same superscript letter(s) within the same column are not significantly different by the Fisher test. According to the Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d) indicate significantly different means within a column.

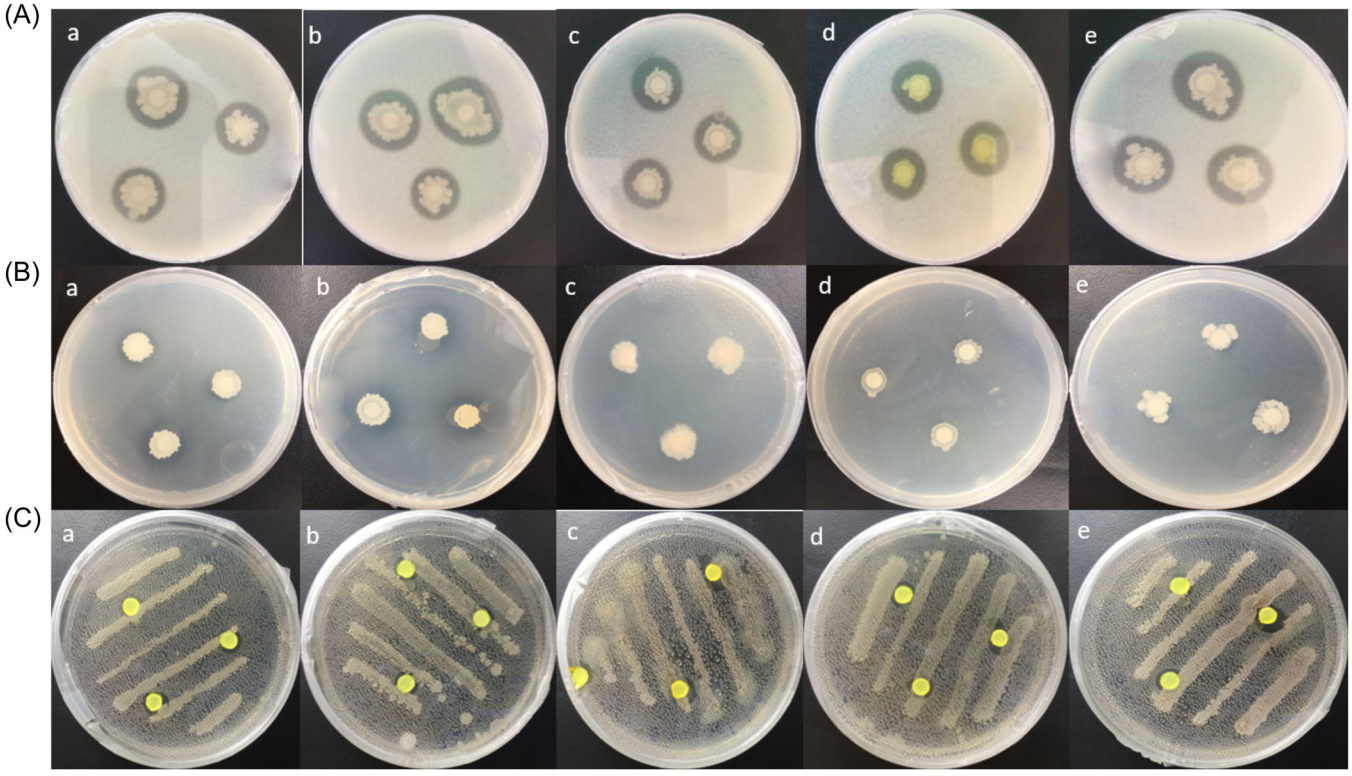

FIGURE 3 Enzymatic bioassay including (A) protease, (B) phosphate, and (C) hydrogen cyanide (HCD) of selected bacterial isolates. (a) M09, (b) M10, (c) M16, (d) M47, and (e) M50.

IAA. The production of secondary metabolites by the isolates varied. For instance, the antagonistic potential of diffusible and volatile metabolites that produce avocado rhizobacterial strains of *Bacillus* has been reported against *Fusarium kuroshium* using a dual plate culture assay [35]. Production of different hydrolytic enzymes is another trait associated with plant growth-promoting rhizobium (PGPR), allowing them to restrict the growth of fungal pathogens that disintegrate their cell wall [11]. The production of chitin lytic enzymes is a biocontrol mechanism related to these bacteria and the essential mechanism [36].

FIGURE 4 In vivo assay of selected bacterial isolates against *Rhizoctonia solani* (RS) in common beans. (A) Plant biometric assay, (B) root mortality assay. (a) Absolute control, (b) M09 + RS, (c) M10 + RS, (d) RS.

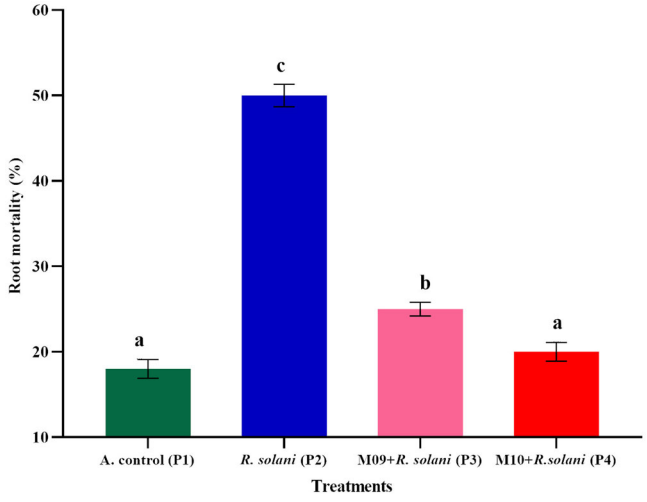

FIGURE 5 Root mortality rate based on the no. of roots in bean plants infected with *Rhizoctonia solani* after 35 days of infection with or without applying bacterial isolates M09 and M10. The "positive control" received only fungal application.

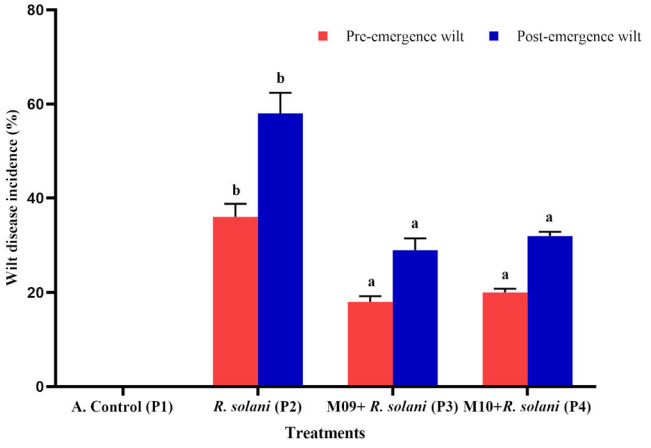

FIGURE 6 The pre- and postemergence wilt incidence based on the no. of bean plants infected with *Rhizoctonia solani* after 35 days of infection with or without the application of bacterial isolates M09 and M10. The "positive control" received only fungal application.

In the pot assay, applying *B. subtilis* M09 and *B. velezensis* M10 considerably suppressed the *R. solani*. The strains showed highly significant, that is, <28.00% of root mortality and pre-emergence wilt incidence in beans against the pathogen. The result agrees with previous findings that reported the ability of *Bacillus* spp. to produce dehydrogenase enzymes in determining the viability of plant survival [14, 29, 37]. Additionally, Idris et al. [38] reported the ability of B. *velezensis* and *B. subtilis* to have lower mortality from root rot in the hyphae of *F. oxysporum*.

The results, the evaluated PGPR considerably improved plant growth in shoot and root elongation and plant biomass content compared with the other treatments. The plant height and biomass increase for bacteria-inoculum may be due to growth regulator hormones [39]. Furthermore, *B. subtilis* and *B. velezensis* have been associated with increased plant biomass and height [40]. Suppression of root pathogens in the

TABLE 4 Effect of selected *Bacillus* strains on the shoot and root elongation.

Treatments	Root length (cm)	Shoot length (cm)	Root weight (g*10)	Shoot weight (g*10)
Absolute control (P1)	10.08 ± 0.03^{c}	13.42 ± 0.12^{a}	3.31 ± 0.05^{c}	12.53 ± 0.42^{ab}
Rhizoctonia solani (P2)	4.28 ± 0.12^{d}	10.57 ± 0.72^{b}	2.44 ± 0.11^{cd}	9.52 ± 0.23^{c}
M09 + *R. solani* (P3)	14.92 ± 0.96^{b}	13.14 ± 0.55^{c}	4.83 ± 0.13^{b}	19.23 ± 0.35^{a}
M10 + *R. solani* (P4)	16.23 ± 0.72^{a}	16.83 ± 0.53^{b}	5.21 ± 0.23^{a}	18.91 ± 0.44^{ab}

Note: The mean values with the same superscript letter(s) within the same column are not significantly different by the Fisher test. According to the Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d) indicate significantly different means within a column.

TABLE 5 Effect of different treatments on the induction of defense enzymes in common bean plantlets.

Treatments	Phenylalanine ammonia-lyase (PAL) (cinnamic acid $h^{-1}g^{-1}$ fresh wet)	Polyphenol oxidase (PPO) (Δ changes in absorbance $min^{-1}g^{-1}$ fresh wt.)	Peroxidase (PO) (Δ changes in absorbance $min^{-1}g^{-1}$ fresh wt.)
Absolute control (P1)	141.08 ± 10.21^{c}	0.015 ± 0.002^{d}	0.047 ± 0.002^{d}
Rhizoctonia solani (P2)	241.13 ± 14.07^{b}	0.021 ± 0.001^{c}	0.080 ± 0.002^{c}
M09 + *R. solani* (P3)	321.04 ± 14.98^{a}	0.075 ± 0.002^{b}	0.142 ± 0.005^{a}
M10 + *R. solani* (P4)	301.09 ± 13.82^{ab}	0.083 ± 0.003^{a}	0.102 ± 0.002^{b}

Note: The mean values with the same superscript letter(s) within the same column are not significantly different by the Fisher test. According to the Fisher's LSD test ($p < 0.05$), different superscript letters (a, b, c, d) indicate significantly different means within a column.

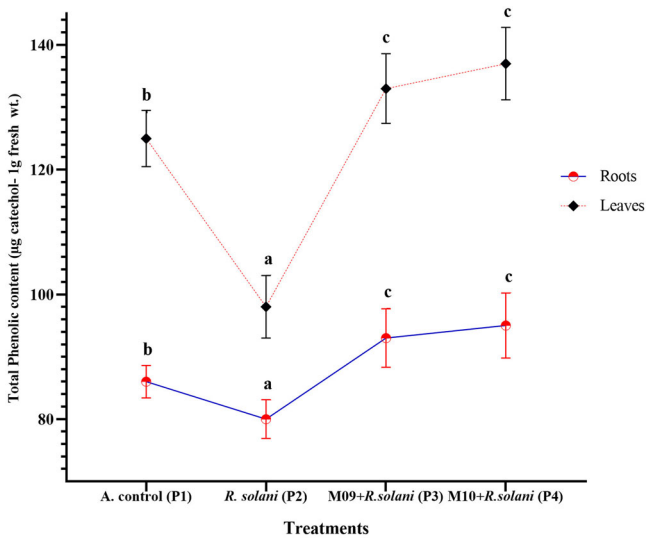

FIGURE 7 The phenolic content based on the roots and leaves in bean plants infected with *Rhizoctonia solani* (RS) after 35 days of infection with or without applying bacterial isolates M09 and M10. The "positive control" received only fungal application.

rhizosphere improves the rooting and growth condition of the host plant. Similar findings have been demonstrated by Paraszkiewicz et al. [41], in which microbe inoculation improved survival, vigor index, shoot/root elongation, and fresh/dry biomasses.

Eliciting the host plant's defense system is another indirect way through biocontrol that makes plants more tolerant of invading phytopathogens. Along these lines, the results demonstrated induction in systemic resistance (ISR) in common beans by antagonistic bacterial strains responding to the pathogen. Without bacterial bioagents, the results showed reduced antioxidant enzymes (PAL, PPO, and PO). A higher phenolic compound was also accumulated in bacterial-inoculated common bean seedlings. This is probably due to the secretion of chitinase and protease, which act as signaling molecules in activating systemic resistance [42]. Numerous studies have shown the ability of PGPR to activate various defense responses in host plant tissue, especially the activity of antioxidant defense enzymes in response to pathogen attacks [43, 44]. Additionally, inoculation with PGPR-activated ISR-related antioxidant enzymes reduced the severity of the disease caused by fungal pathogens [45]. In our present study, we described a good antagonistic potentiality of *Bacillus* spp. from Lake Magadi in controlling *R. solani* in common beans.

It was concluded that *Bacillus* spp. from Lake Magadi had significant antagonistic activity against *R. solani*. Additionally, they displayed antifungal volatile and nonvolatile compounds production in suppressing mycelium growth. In pot assay, *B. subtilis* M09 and *B. velezensis* M10 showed significant disease control and production of antioxidant enzymes and phenolic content. Therefore, present a putative biocontrol agent for managing fungal pathogen in common beans.

AUTHOR CONTRIBUTIONS

Tofick B. Wekesa: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; supervision; visualization; writing—original draft. **Eliud N. Wafula**: Data curation; investigation; supervision; validation; writing—review & editing. **Ndinda Kavesu**: Investigation; validation **Robert M. Sangura**: Validation, writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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