Identification and Biocontrol of Two New Soft Rot Bacteria Isolated from Stored Potatoes in Iraq

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Abstract:

This investigation aimed to isolate and identify the bacterial strains responsible for the soft rot disease in potato tubers collected from various locations across Duhok province, Iraq. Two Gram-negative, A motile bacillus (*Providencia rettgeri*, PDPRE) and a non-motile bacillus (*Klebsiella michiganensis*, PDKMI) were identified for the first time in Iraq as the causal agents of soft rot in stored potatoes based on morphological, biochemical, and molecular methods, including 16S rRNA gene sequencing. In vitro antibacterial screening of plant extracts and bioagents using the agar well diffusion method revealed that *Pseudomonas* sp. and Myrtle extracts demonstrated the highest inhibition zones at 50% concentration. In vivo testing on potato tubers further validated their antagonistic potential, with *Bacillus subtilis* and *Pseudomonas fluorescens* showing the greatest reduction in disease severity, reducing infection levels to 23.2% and 23.23% for *K. michiganensis* and 20% and 26.67% for *P. rettgeri*, respectively. Overall, *P. rettgeri* exhibited greater susceptibility to treatments. These findings highlight the significant impact of these pathogens on potato quality and market value and support the potential of natural bioagents and plant extracts over chemical treatments for managing soft rot in post-harvest potatoes.

Keywords: Potato, *Providencia rettgeri*, *Klebsiella michiganensiss*, PCR, Bioagents, Biochemical tests

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Introduction

Potato (*Solanum tuberosum* L.) is one of the most significant agricultural products in the world. It is a rich source of nutrients that can be included in a nutritious diet. Potatoes rank as the third most important food crop for human consumption worldwide, following rice and wheat consumption. after the rice and wheat coming in second and third respectively [16]. [20] Diseases caused by these pathogens lead to significant economic losses during crop growth, harvest, and storage. directly reducing global crop yields [5, 60]. Soft rot is caused by several bacterial species, Klebsiella species can cause infections in plants as well as both humans and animals (25,42)

K. variicola is associated to a number of plant diseases such as bacterial soft rot of carrots in [India [14], rhizome rot of bananas in India [37], and necrotic soft rot of bananas in Haiti [23]. However, although reports of Klebsiella variicola infections in plants are limited, most strains identified so far have been isolated from clinical and environmental sources.

In recent years, Klebsiella strains have been involved in plant diseases progressively [21,29]. A pathogenic bacterium (*Klebsiella michiganensis*) has been associated with Mulberry (*Morus alba L.*) wilt disease in Guangxi, China, and the entire genome sequence of *K. michiganensis* AKKL-001 was isolated from diseased mulberries [39].

Providencia species are Gram-negative, urease-producing bacteria that are members of the Enterobacteriaceae family. Although these species are found in the environment, they can act as opportunistic pathogens in humans, and can also be found in waste and rivers [56,6]. These could account for their isolation from various foods and food items [58,4,46]. Recently, *Providencia rettgeri* was identified as a plant pathogen that cause soft rot disease in calla lily plants, causing serious threats in the commercial production [62]. Another study conducted by [65] classified *P. rettgeri* as the causal agent of brown slime flux of *Populus tomentosa* tree in China.

Molecular identification includes amplifying extracted DNA from bacterial isolates using the polymerase chain reaction (PCR) under specific thermal cycles. That is accomplished after DNA extractions, amplification using specific primers, and sequencing [57,64]. For bacterial taxonomic identification, the 16S rRNA and the RNA polymerase beta subunit (rpoB) genes are commonly used as molecular markers [7]. Biological control techniques have gained popularity as viable, sustainable, and eco-friendly plant disease management options throughout the last 20 years [26].

Chemical bactericides are not advised for controlling soft rot bacteria. They are costly, short-lived, harmful to the environment, and promote bacterial resistance, biological control using Trichoderma, Bacillus, and Pseudomonas species is considered an effective strategy against bacterial soft rot and other soil-borne pathogens. Bacillus spp. suppresses plant diseases through broad-spectrum antibiotic and enzyme production, while non-pathogenic Pseudomonas spp., as plant growth-promoting rhizobacteria (PGPR), inhibit pathogens in the root zone and enhance plant growth [8,32,45]. Based on Trichoderma functions as competition, iron-chelating metabolites, plant resistance inducers, and antibiotics, more than 180 secondary metabolites of Trichoderma have

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been classified into several chemical compounds [61].

P. carotovorum can be suppressed by a range of plant extracts, according to recent research. Green tea, for instance, significantly decreased potato tuber maceration and totally suppressed the pathogen in vitro . Similarly, ethanolic peel extracts of *Punica granatum* (pomegranate) were shown by [19]to totally prevent soft rot on potato tubers (100% treatment efficiency, with no illness seen for 14 days). According to, oils derived from thyme, oregano, cinnamon, mint, and other herbs each created sizable inhibitory zones and considerably decreased tuber rot in vivo.nticable inhibition of a pathogen's mycelial growth and its sporulation was demonstrated in vitro experiment by [53] to assess the effectiveness of Myrtle leaf extracts (*Myrtus communis*) on the growth and sporulation of *Fusarium culmorum*, the cause of wheat damping-off disease, when cultivated on PDA, which showed a 30.06% inhibition of mycelial growth.

It has been claimed that applying plant extracts such as Eucalyptus oil immediately destroyed 11 fungal strains and 22 tested bacterial strains, demonstrating the oil's nematocidal, insecticidal, and pesticidal properties [48]. Since ancient times, people have been using the annual plant Myrtus communis L. for food, medicinal, and spice reasons; the leaves include volatile oils, tannins, and flavonoids such as myricetin, catechin, and quercetin derivatives[50].

The objectives of the study were to isolate and detect the bacterial pathogens responsible for soft rot in potato tubers using molecular techniques, to evaluate bacterial bioagents for their effectiveness against soft rot pathogens, and to assess the potential of various plant extracts in controlling these pathogens.

Material and Method:

Sample Collection

A survey for eight different field locations in Duhok province (Goran, Peshabir, Kalakgi, Bardarash, Domiz, Fahidy, Rashdiye, and Qasroke), grocery store vegetables, and cold storage facilities in Qasroke, were also used for collecting potato tubers that showed signs of soft rot disease, including discoloration and tissue decay. the sampling was carried out from late August until early September in 2024. Each tuber sample was placed in a sterile plastic bag and labeled with relevant details, including the date of collection, sample type, size, and location. Upon arrival at the laboratory, the samples were stored at 4°C until further analysis was conducted.

Isolation of the Causal Pathogen:

Tuber samples were collected from the field of the same location and various grocery vegetable and storage facilities in the province of Duhok the samples were surface-sterilized for three minutes using a 5% sodium hypochlorite solution containing free chlorine, The tubers pieces were peeled and then sliced into tiny pieces ranged in length from 0.5 to 1 cm. for bacteria isolation. Then these pieces were dried on sterile filter paper. The parts were crushed using a ceramic pestle with distilled water. These were then transferred to sterile Petri plates containing nutrient agar. To prepare a bacterial suspension, 5 mL of distilled water was added while continuously stirring. Using a sterile inoculation loop, a portion of the resulting suspension was collected and streaked onto sterile 9 cm Petri dishes containing nutrient agar (NA). The bacterial

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isolates were then stored at –20°C in slant tubes using a 70% glycerol solution for preservation [3,27].

Preparation of soft rot bacteria inoculum

Each strain of bacteria was cultured in slant tubes on the nutrient agar (2%) medium to create the bacterial inoculum. After that, the inoculation tubes were incubated for 48 hours at 28 °C ± 2. A bacterial suspension was prepared by carefully removing the bacterial growth from the agar surface and scraping it into 5 mL of sterile 0.2 M phosphate buffer (pH 7.2). The density of the bacterial inoculum was adjusted to approximately 10^8 colony-forming units (CFU)/mL using the serial dilution method. The samples were rechecked within 12 hours after being stored at 4 °C [44].

Morphological and Microscopic Features of the Bacterial Isolates

Blood, MacConkey, and nutrient agar were used for growing and macroscopic description of the two bacterial isolates (PDPRE and PDKMI) After being stained, and the characteristics of the bacterial colonies were studied including the type of movement, cell shape, edge form, spore formation, and gram staining, following the methods described by [55].

Biochemical tests

According to the procedures outlined by [12], the bacteria causing soft rot were diagnosed based on the biochemical assays, which are:- Gram staining, Catalase test, Oxidase test, (TSI) agar test, and Indole production in addition to their ability to grow at 37°C.

Molecular identification

1. DNA Extraction

DNA was extracted from bacterial cultures using the Beta Bayern Tissue DNA and Bacterium Preparation Kit (Beta Bayern GmbH, 90453 Bayern, Germany). A NanoDrop spectrophotometer was used to measure the extracted genomic DNA's concentration and purity. Following [51] protocol, DNA integrity was verified by electrophoresis on a 1.0% agarose gel prepared in 1X Tris-Acetate-EDTA (TAE) buffer and stained with ethidium bromide (0.5 µg/ml). The DNA samples were kept at -20°C.

2. Polymerase Chain Reaction (PCR)

A total of Each PCR reaction was carried out in a total volume of 25 µL, containing 2× Taq DNA Polymerase Master Mix (AMPLIQON A/S, Stenhuggervej 22), 10 pmol of each primer, DNase-free water, and template DNA. The amplification was performed targeting the 16S rRNA gene using universal primers 27F (5′AGAGTTTGATCCTGGCTCAG-3′) 1492R (5‘GGTTACCTTGTTACGACTT-3′) [22]. Each PCR reaction included 21 µL of nuclease-free water, 1 µL of each primer (10 µM), and 2 µL of genomic DNA. The thermal cycling steps included an initial 5-minute denaturation at 94 °C, 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for a minute, extension at 72 °C for 30 seconds, and a final extension of 72 °C for 7 minutes.

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3. Sequence alignment and phylogenetic analysis

The PCR products for the partial 16S rRNA gene were sequenced using the ABI Prism Terminator Sequencing Kit (Applied Biosystems) at Macrogen Molecular Company in Korea. The base calls were reviewed and edited using the FinchTV software program. The NCBI GenBank dataset was used to compare and align the query sequences with related bacterial sequences and identify the closest matches. The 16S rRNA gene sequences were aligned using the ClustalW algorithm [34]. The phylogenetic tree was then constructed using MEGA 11 Molecular Evolutionary Genetics Analysis Version 7.0's maximum parsimony method to perform a divergence analysis among these sequences [59]. A bootstrapped reliability test with 1000 repeats will be used to assess the estimated phylogenetic tree [54].

Evaluation of Plant Extracts and Bioagents Against the Causal Pathogen

1: Preparation of the Biocontrol Agents

In this study, (*Bacillus subtilis* and *Pseudomonas fluorescens*) and (*Trichoderma harzianum*) obtained from the Mycology Bank and Plant Protection Department at the University of Duhok were tested. Culture filtrates of each bacterial biocontrol agent were prepared in sterilized nutrient glucose broth (2%) following the method of [1]. Culture filtrates of *Trichoderma harzianum* were prepared in 250 ml flasks containing sterilized 2% potato glucose broth, according to the method described by [1].

2: Preparation of Aqueous Plant Extracts:

Plant leaf extracts were prepared from *Myrtus communis* (Myrtaceae), and *Eucalyptus camaldulensis* (Myrtaceae). The aqueous plant extracts were prepared at concentrations of 10%, 25%, and 50% (w/v) by mixing 10 g, 25 g, and 50 g of powdered plant material with 100 mL of sterile distilled water, respectively. The filtered extracts were stored in sterile glass bottles at 4°C in a refrigerator [33].

In Vitro Antibacterial Activity of Plant Extracts and Bioagent Filtrates Using the Agar Well Diffusion Method:

The antibacterial activity of plant or *Trichoderma harzianum* extracts was applied using the agar well diffusion method of [9]. Each well was filled with 100 µL of plant extract or bioagent filtrate at concentrations of 0%, 10%, 25%, or 50%. To ensure replications, the experiment was conducted using six plates for each concentration, with each plate containing three wells. The inhibition zone was measured after 24 hours of incubation at 30°C [9].

In Vivo Assay of Bioagents and Plant Extracts on Potato Tubers:

The *Solanum tuberosum* cv. Arizona tubers were used to test the inhibitory effects of a 50% concentration of plant extracts and culture filtrate of bioagents against *Providencia rettgeri* and *Klebsiella michiganensis*. Surface sterilized Potato tubers were treated with culture filtrates or plant extracts and air-dried for two hours. They were then inoculated with the bacterial suspension and left to dry again before being stored at 30 ± 1 °C in sterile plastic bags. Negative control treatment includes spraying

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the potato with sterile distilled water only while the positive control includes the pathogen only. The experiment was conducted using a factorial complete randomized design (CRD), with five replications assigned to each treatment. The disease's severity was determined after four weeks of treatment using the scale suggested by [10]. The degree of tuber rot was 0: No symptoms of rot, 1: 1-15% tuber rot, 2: 16-30% tuber rot, 3: 31-45% tuber rot, 4: 46-60% tuber rot, 5: ≥61% tuber rot. The Tuber rot severity was calculated using the following formula: $V = \sum(nv \times v) \times 100/N \times G$, where V= disease score, n = number of tubers showing a particular score, N= Total number of tubers assessed, and G= highest score 5.

Results

Isolation and Morphological Characteristics of the Investigated Bacteria

In the present research, two bacterial strains, *Providencia rettgeri* (PDPRE) and *Klebsiella michiganensis* (PDKMI), were isolated for the first time in Iraq and diagnosed from soft rot tubers collected from three different locations. During the post-harvest storage, these bacteria caused odors and a significant decrease in the quality of infected tubers. Due to increased rotting and a reduction in market value, their presence resulted in economic losses. The isolates were identified as *Klebsiella michiganensis* and *Providencia rettgeri*, both Gram-negative, and rod-shaped bacteria. *K. michiganensis* colonies appeared as dry, creamy white to off-white and non-mucoid, consistent with previous descriptions [43]. These species have been previously isolated from various environments, including soil, water, food, plants, and insects [17, 39,28] (Figure 1).

Figure 1: The colony growth of *Providencia rettgeri* (left) and *Klebsiella michiganensis* (right) on the three different culture media (Nutrient Agar, MacConkey Agar, and Blood Agar).

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Biochemical tests

According to the biochemical reaction, the two isolates (PDKMI and PDPRE) were identified as *Klebsiella michiganensis* and *Providencia rettgeri*, respectively. Both isolates are Gram-negative bacteria with morphological features that match the characteristics of *Providencia rettgeri* and *Klebsiella michiganensis*, which were described previously. The development of bubbles upon the addition of hydrogen peroxide indicated that both isolates showed a positive result in the catalase test. *P. rettgeri* gave a negative oxidase test because no color changes occurred, while *K. michiganensis* gave a positive test and a rich purple color occurred, indicating it does produce the enzyme cytochrome c oxidase. In the (H_2 S) generation test, both bacteria are negative because no black precipitate was seen. Both isolates displayed yellow coloration in the slant and/or butt of the Triple Sugar Iron (TSI) agar test, indicating the production of acid as a result of the fermentation of carbohydrates. At an incubation temperature of 37°C, both bacterial strains demonstrated growth. Additionally, the indole test's red ring indicated that *Klebsiella michiganensis* and *Providencia rettgeri* were both positive for indole production (Table 1). The results are similar to the previous studies [52,2].

Table 1: Biochemical and Physiological Characteristics of *Klebsiella michiganensis* and *Providencia rettgeri*

Bacterial	Oxidase test	Gram staining	H2S	Triple sugar iron	Indole production	Catalase test	Growth 37°C
Klebsiella michiganensis	Positive	Negative	Negative	Positive	Positive	Positive	Positive
Providencia rettgeri	Negative	Negative	Negative	Positive	Positive	Positive	Positive

Molecular Detection of Bacterial Isolates:

The Nanodrop spectrophotometer measured the concentration of DNA between 200 ng/µl to 300 ng/µl with good purity, which ranged from 1.6 to 1.8. The amplification of DNA targeting the 16S rRNA gene showed a distinct single band of 1420 bp on agarose gel for *Klebsiella michiganensis* and *Providencia rettgeri* as shown in Figure 2. According to the NCBI database, Blast analysis revealed a high degree of similarity of the isolate PDPRE with the accession

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number (PV425928) to the *Providencia rettgeri*. While the isolated PDKMI with accession number (PV424661) showed the highest DNA sequence similarity to *Klebsiella michiganensis*

Phylogenetic analysis

According to the NCBI database, isolate PDPRE with the accession number (PV425928) has the highest query DNA sequences that are similar to those of *Providencia rettgeri*. PDPRE isolate clustered with sequences of related species to form a distinct species group supported with a high bootstrap value (up to 97%) according to the maximum parsimony phylogenetic tree with 15 references, including the one in this study Figure 3.

PDKMI with accession number (PV424661 also showed the highest DNA sequence similarity to *Klebsiella michiganensis i*n the BLAST results. This species is grouped with high bootstrap support (up to 99%) in the phylogenetic tree constructed using maximum parsimony with 15 reference sequences reflecting its close taxonomic relationship with the same species in the NCBI dataset, including USA OR831660, IRAQ PP179211, INDIA OR136362, CHINA ON556591, ITALY PP779874, and CHINA PP512815.

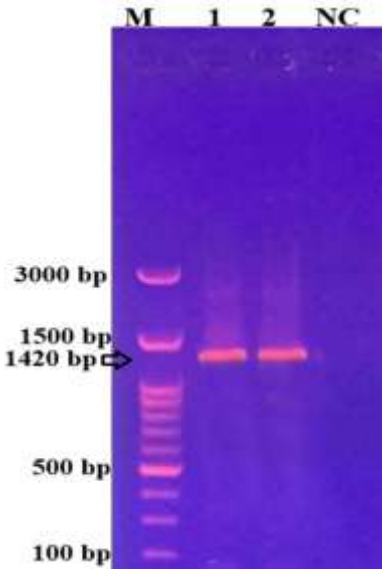

Figure 2. Agarose gel electrophoresis of PCR amplification of partial 16S rRNA gene, wells include M; Ladder (3000_100 bp), lane1, *Providencia rettgeri* gene band, Lane2; *Klebsiella michiganensis* gene band with the size of 1420 bp.

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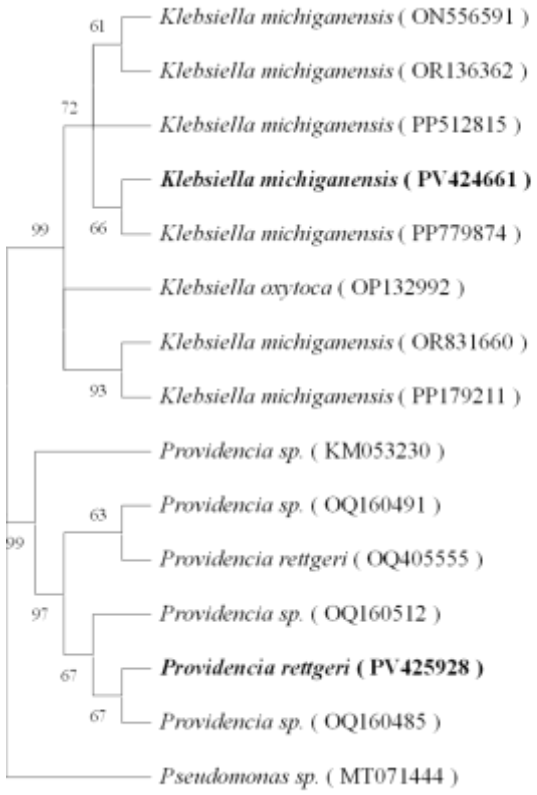

Figure 3. Phylogenetic maximum parsimony analyses of ITS were generated using MEGA 11 for two new bacterial species *Providencia rettgeri* (PV425928) and *Klebsiella michiganensis* (PV424661), on potato tuber. The tree was rooted in *Pseudomonas* sp.

In Vitro Antibacterial Activity Using

Agar Well Diffusion Method:

Across all treatments, there was a clear concentration-dependent increase in antibacterial activity, with 50% concentration consistently yielding the largest inhibition zones (Figure 4) and (Table 2). The highest significant inhibition results were recorded at a 50% concentration of the *Pseudomonas fluorescens* filtrates, producing inhibition zones measuring 26.43 mm against *Klebsiella michiganensis* and 24.83 mm against *Providencia rettgeri*. The significant inhibition was also recorded with Myrtle extract at the same concentration (50%) resulted in inhibition zones 24.93 mm for *Klebsiella michiganensis* and 25.20 mm for *Providencia rettgeri*, respectively. The Results of Eucalyptus extracts gave 18.3 mm inhibition against *Klebsiella michiganensis* and highly significant inhibition (25.03 mm) against *Providencia rettgeri*. *Bacillus subtilis* filtrate treatments showed moderate antibacterial effects, with inhibition zones at 50% concentration (19.63mm) against *Klebsiella*

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michiganensis. *Trichoderma harzianum* filtrates also gave moderate inhibition zones measuring 16.30 mm against *Klebsiella michiganensis* and 21.68 mm against *Providencia rettgeri*, respectively. Across both pathogens, *Providencia rettgeri* appeared more susceptible to the treatments, consistently showing slightly larger inhibition zones than *Klebsiella michiganensis*, particularly at higher concentrations. the interaction between the two factors (bioagents and plant extracts) with the best inhibition zone against the pathogens were *Pseudomonas fluorescens* and Myrtle, as the best with the highest inhibition zone (14.28mm, 14.61mm, respectively), the third place for *Bacillus subtillis*, and the fourth was for the Eucalyptus extract the fifth was for the *Trichoderma harzianum* bioagent.

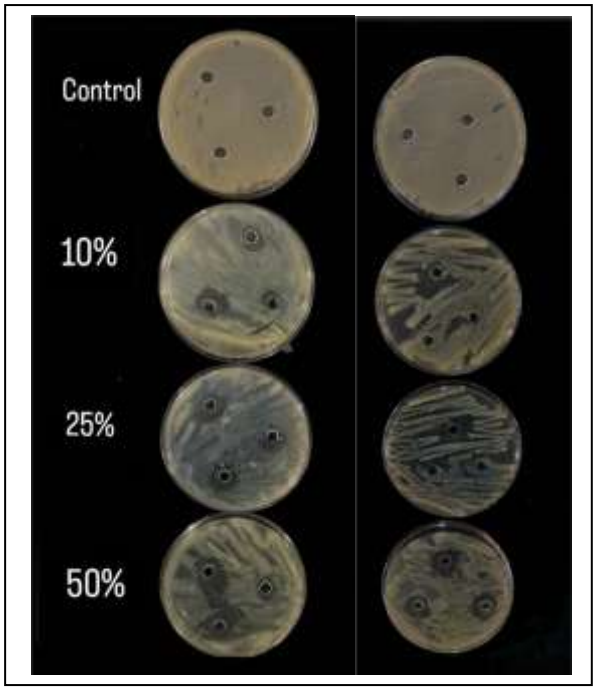

Figure 4. Effect of *Pseudomonas fluorescens* filtrates as antibacterial Activity Against *Providencia rettgeri* (left) and *Klebsiella michiganensis* (right)

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Table 2. Effect of Different Biological Treatments and Concentrations on Antibacterial Activity Against *Klebsiella michiganensis* and *Providencia rettgeri*

Bacteria	*Treatment*	*Inhibition zone (mm)*		
		10% Conc.	*25% Conc.*	*50%Conc.*
Klebsiella michiganensis	*Eucalyptus Extract*	9.37 q-s	11.40 n-r	18.03 c-g
Providencia rettgeri		15.80 e-k	19.30 b-d	25.03 a
Klebsiella michiganensis	*Myrtle Extract*	12.93 j-p	21.17 b	24.93 a
Providencia rettgeri		14.53 h-n	18.10 c-f	25.20 a
Klebsiella michiganensis	*Trichoderma harzianum*	2.50 t	7.40 s	16.30 d-i
Providencia rettgeri		12.95 k-p	15.90 e-k	21.68 b
Klebsiella michiganensis	*Pseudomonas fluorescens*	14.43 h-o	18.83 b-e	26.43 a
Providencia rettgeri		12.33 l-q	17.33 c-h	24.83 a
Klebsiella michiganensis	*Bacillus subtilis*	13.63 i-o	14.87 g-m	19.63 bc
Providencia rettgeri		11.33 o-r	16.17 e-j	26.33 a

*Means with the same letter are significantly different according to Duncan's Multiple Range Test (P=0.05)

In Vivo Assay of Bioagents and Plant Extracts on Potato Tubers.

The data show that all treatments significantly reduced disease compared to the control, which had 100% disease severity for both pathogens, confirming their antagonistic potential. *Bacillus subtilis* and *Pseudomonas fluorescens* were the most effective treatment, showing the lowest significant disease severity, 23.20% and 23.23% against *Klebsiella. michiganensis*, and 20.00% and 26.67% against *Providencia rettgeri,* respectively. *Myrtle*

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communis extracts showed moderate control, reducing disease severity to approximately 42.20 % and 26.43% against *Klebsiella michiganensis* and *Providencia rettgeri,* respectively. Comparing pathogen response, *Providencia rettgeri* was more effectively suppressed overall, with a lower mean disease level (39.1%) compared to *Klebsiella michiganensis* (48.4%), indicating some degree of pathogen-specific variation in treatment efficacy (Table 3, Figure 5).

Table 3. Effect of Biological Treatments on Disease Severity Caused by Klebsiella michiganensis and Providencia rettgeri in Potato Tuber Lesion and Damage after 4 Weeks

Treatments (50% Con. Filtrates)	*Klebsiella michiganensis*	*Providencia rettgeri*	Effect of treatments
Eucalyptus sp.	39.97 ab	39.97 ab	39.97 abc
Myrtle communis	42.20 ab	26.43 ab	34.32 abc
Trichoderma harzianum	55.33 b	26.43 ab	40.88 bc
Pseudomonas fluorescens	23.23 a	26.67 ab	24.95 ab
Bacillus subtilis	23.20 a	20.00 a	21.60 a
control	100.0 c	100.0 c	100 d
Effect of Bacteria	48.4 a	39.1 b	******

*Means with the same letter are significantly different according to Duncan's Multiple Range Test (P=0.05)

This result is confirmed in Figure 6, which illustrates the order of treatments based on their effectiveness in reducing disease severity; According to the statistical analysis, *Bacillus subtilis* and *Pseudomonas fluorescens* were the most effective biocontrol agents, showing the lowest disease severity (21.60% and 24.95%, respectively. Myrtle treatment demonstrated moderate levels of disease suppression (34.32%).

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Figure 5. Effect of biological agents and plant extracts on reducing disease Severity in Potato tubers after four weeks of infection by *Klebsiella michiganensis* (right) and *Providencia rettgeri* (left), with complete rotting of tubers in the control treatment one week after inoculation

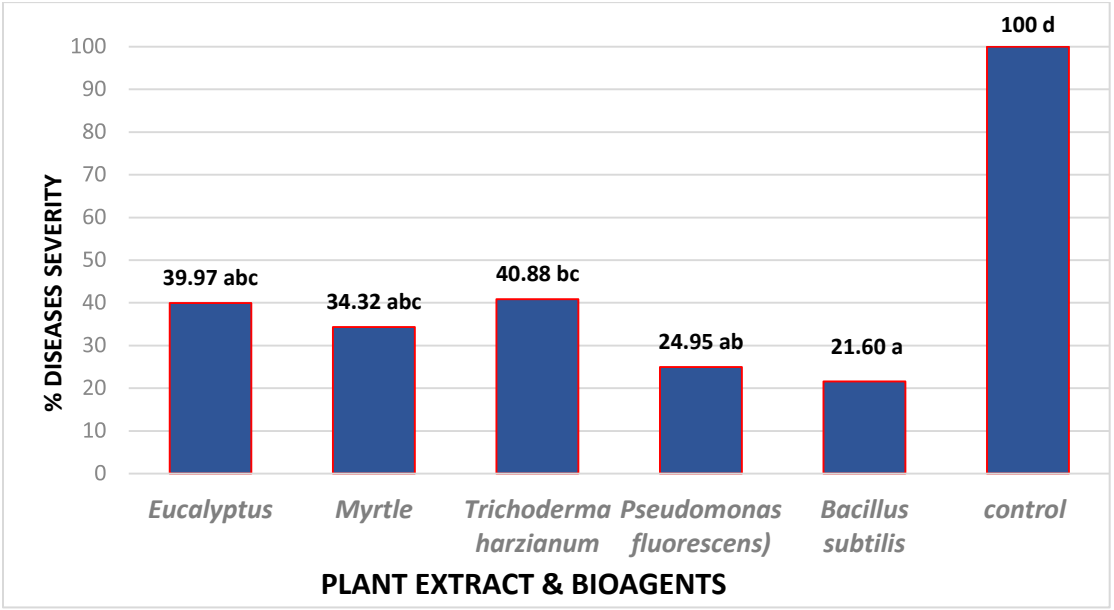

Figure 6: Effect of Biological Agents and Plant Extracts on Reducing Disease Severity in Potato Tubers after 4 weeks of treatment.

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Discussion

Soft rot is a common disease of many vegetables and crops during storage and transportation. The main bacteria that cause this infection are Pectobacterium [39,41,62]., However, two new bacteria also cause soft rot diseases, including *Klebsiella michiganensis* and *Providencia rettgeri* . presented in this study have been recorded to cause potato soft rot diseases. The high humidity and inadequate ventilation play an important role in the spread of soft rot from healthy tubers to infected ones, according to a study conducted by [18] on the storage conditions of *Dickya dianthicola* and *Pectobacterium carotovorum*. Our results confirmed the variation of infection between the two bacterial species and showed that *P. rettgeri* is more virulent and its symptoms appeared earlier than *K. michiganensis*. This variation might be due to genetic diversity among pathogens, to different enzyme production, and to cultivar susceptibility, as proved by [15]. In vitro results of this study confirmed the high efficiency of the bioagent *Pseudomonas fluorescens* and the Plant extract of Myrtle against the tested bacteria. This finding agrees with [49], who highlight the role of *Pseudomonas fluorescens* in producing different kinds of molecules (antibiotic compounds, lytic enzymes, lipopeptides, and sediphores involved in antagonist interaction with other organisms. According to[13], myrtle essential oil is highly effective against two Pectobacterium species, *P. aeruginosa* and *P. carotovorum.*

The aggressive nature of *Providencia rettgeri* and *Klebsiella michiganensis* under storage conditions was confirmed by the in vivo evaluation using potato tubers, with untreated controls showing 100% disease severity. By secreting enzymes that break down plant cell walls, such as pectinases and cellulases, these pathogens spread quickly through contact and moisture in storage environments, allowing for tissue maceration and bacterial colonization. Cross-infection is more likely to occur in tuber storage due to the enclosed, humid conditions, particularly through harvesting or handling wounds.

Both *Pseudomonas fluorescens* and *Bacillus subtilis* significantly reduced disease severity down to 20–26% demonstrating strong antagonistic activity in vivo. Their efficacy likely stems from multiple mechanisms, including the production of antimicrobial metabolites, competition for nutrients and colonization sites, biofilm formation on tuber surfaces, and induction of host defense responses [63,47,11,35]. These actions collectively inhibit pathogen establishment and slow the progression of soft rot in stored tubers. Antibiotic activity against the Xcc was also demonstrated by *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus velezensis*, Paenibacillus, and *Bacillus thuringiensis* [38,24 ,36 ,31]. Furthermore, the kind of bacteria, the type of culture media, and the mean diameter of the inhibitory zone all showed significant connections [30,60]. GC-MS and HPLC analysis of two Bacillus strains revealed several antimicrobial compounds, including volatile organics (alkenes, benzenes, carboxylic acids, indoles, pyrazines) and bioactive metabolites like lipopeptides and antibiotics, present in benzene and ethyl acetate extracts [31]. The production of iturin, kurstakin, bacillomycin, and surfactin plays a key role in fighting bacterial infections, with these compounds closely linked to enhanced disease resistance [40].

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Conclusion

Providencia rettgeri and *Klebsiella michiganensis* were morphologically and molecularly identified for the first time in Iraq as the causes of soft rot bacteria in potato tubers at Duhok province. The effectiveness of Pseudomonas fluorescens, Bacillus subtilis, and myrtle extract significantly inhibited the growth of these bacteria during in-vitro test and decreased the disease severity in potato tubers when inoculated with these two bacteria. This finding emphasizes including the bioagent and plant extracts in the post-harvest control strategies, offering an alternative to synthetic fungicides. These natural methods are being explored as more environmentally friendly and sustainable options.

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