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Native bacterial bioagents for management of potato soft rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum*

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Abstract

Background The reduced rate of potato production may occur under different conditions such as field, transit, storage, and marketing. Potato cultivation is frequently affected by various pathogens, among which *Pectobacterium carotovorum* subsp. *carotovorum* is a notorious bacterial pathogen responsible for causing bacterial soft rot disease. This pathogen poses a significant threat to potato production worldwide, resulting in substantial economic losses and food security concerns. This study aimed to investigate the effectiveness of three bacterial bioagents, namely *Brevundimonas bullata, Bacillus siamensis*, and *Bacillus velezensis*, against *P. carotovorum* subsp. *carotovorum*, a notorious bacterium responsible for causing potato tuber soft rot disease.

Results Fifteen isolates were isolated from rhizosphere of potato plants. Out of 15 isolates, 3 isolates, No. 1, 11, and 12, showed highly antagonistic property to control the growth of *P. carotovorum* subsp. *carotovorum* in vitro. They were identified as *B. bullata, B. velezensis*, and *B. siamensis*, by using 16S rRNA nucleotide sequence analysis. Results from these experiments revealed that three bioagents exhibited notable inhibitory effects on the growth of the pathogenic bacterium. However, *B. velezensis* stood out as the most effective inhibitor among the tested bioagents, showcasing the highest level of bacteriostatic activity (2.6 cm). The findings from the in vitro experiments provided promising insights into the potential of these bacterial bioagents as natural antagonists against potato tuber soft rot disease. Building upon these promising results, the study recommended the utilization of all tested bioagents for controlling soft rot disease in potato tubers, especially during storage conditions where the risk of bacterial proliferation is heightened. Treatment with *B. bullata*, *B. siamensis*, and *B. velezensis* demonstrates varying degrees of efficacy in controlling the progression of soft rot disease. Notably, *B. velezensis* shows promising results with relatively low disease severity scores across all time points compared to the other treatments. *B. velezensis* demonstrates the lowest disease severity percentage at 7.00% compared to the other treatments.

Conclusions The study underscores the significant potential of *B. bullata*, *B. siamensis*, and *B. velezensis* as effective biological control agents against potato tuber soft rot disease. By targeting the causative agent directly, these bioagents offer a promising avenue for mitigating the detrimental impact of soft rot disease on potato production, thereby contributing to improved crop yield and quality.

Keywords Soft rot disease, Potato, Bioagents, Antibacterial, Greenhouse

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Background

Pectobacterium carotovorum subsp. *Carotovorum(Pcc)* is a highly destructive phytopathogen known for its ability to rapidly break down plant cell walls, leading to the characteristic soft rot symptoms in potato tubers. The bacterium's capacity to survive in soil and on plant debris and its wide host range make it a challenging target for disease management strategies (Doolotkeldieva et al. 2016). Soft rot disease of potato tubers caused by *Pcc* causes a significant reduction in yield, resulting in economic losses in the field during storage and transit, causing losses of up to 60% (Sulaiman et al. 2020). Traditionally, managing bacterial diseases in crops has relied on chemical pesticides and cultural practices. While these approaches have demonstrated some efficacy, they come with inherent challenges. Pesticide overuse raises concerns about environmental impact, resistance development in target pathogens, and potential harm to non-target organisms. Moreover, the adaptability of *P. carotovorum* subsp. *carotovorum* to varying environmental conditions complicates the success of conventional control methods (Hossain et al. 2019).

In response to the limitations of conventional approaches, a paradigm shift toward sustainable and eco-friendly strategies has gained momentum. Biological control, harnessing the power of nature's defenses, holds promise as a viable alternative for managing bacterial pathogens. This approach leverages the antagonistic interactions between organisms to suppress or eliminate the target pathogen, providing a sustainable and environmentally sound solution to disease management. Biological control is particularly compelling in the context of Pcc, given the challenges associated with chemical control and the ecological implications of its widespread use (Azaiez et al. 2018). The intricate relationships within the plant microbiome involving beneficial microorganisms present an untapped resource for developing effective biocontrol strategies (Abd El-Wahed et al. 2023; Idrissi et al. 2021). By unraveling the mechanisms of biological control against Pcc, we can unlock novel approaches that mitigate the impact of potato soft rot and contribute to the broader understanding of microbial ecology in agricultural systems (Czajkowski et al. 2015).

This study aimed to address the urgent need for effective and environmentally friendly solutions to control bacterial soft rot in potatoes. An evaluation of several bacterial bioagents as potential alternatives for managing *P. carotovorum* subsp. *carotovorum* infections was presented. Comprehensive laboratory and storage studies were presented to assess the efficacy of these bacterial bioagents in mitigating disease severity and their potential to serve as sustainable tools for the protection of potato crops.

Methods

Source of pathogen and Preparation of bacterial suspension

The high pathogen isolate of *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc14), MT510006, was isolated and identified by the authors. To prepare the bacterial suspension of Pc14, a pure bacterial culture was grown in 100 ml of nutrient sucrose broth (NSB) and then incubated for 48 h at 27 ± 2°C on a rotary shaker at 150 rpm. Subsequently, the resulting bacterial suspension was centrifuged at 7000 rpm for 3 min. The pellet was then re-suspended in sterilized water, and the bacterial suspension was adjusted to a concentration of 1×10^8 colony-forming unit (cfu/ml) using a spectrophotometer (Milton Roy Company–Spectronic 20D) at a wavelength of 620 nm, following the method described by McGuire and Kelman (1984).

Isolation of bioagents

Soil samples collected from potato fields at Assiut Governorate, Egypt and Hada-al-Sham, were used to isolated native bacterial bioagents. The isolation process followed the protocol outlined by Gaete et al. (2020). Initially, soil samples were cleaned of root debris, plants, and other impurities. Two grams of soil was then mixed with 4 ml of sterilized distilled water and thoroughly vortexed. Serial dilutions ranging from 10^4 to 10^9 were prepared from the supernatant. From each dilution 100 µl was streaked onto nutrient sucrose agar (NSA) medium plates using a sterilized bacterial rod. Plates were subsequently incubated at 30 °C for 24 h, following the method described by Urrea et al. (2011). Bacterial colonies were purified on NSA medium, and the purified isolates were preserved at 4 °C.

Agar disk diffusion method

The potential of the isolates to antagonize against Pcc14 in vitro was assessed using the method described by Sallam et al. (2021). The bacterial isolates were cultured in nutrient agar (NA) medium for two days at 27 °C. A 100 µl inoculum (10^8 CFU/ml) of a 48-h-old culture of Pcc14 was spread evenly on the medium to create a lawn for each isolate separately, with three replications, and each replication consisting of five plates. Subsequently, using a sterile corn borer, three wells of 5.0 mm in diameter were formed on each Petri plate. A 50 µl inoculum (10^8 CFU/ml) of each biocontrol isolate was introduced into each well separately. The Petri plates were then incubated at 27 °C for 2 days. The formation of a zone of inhibition by the bioagent strains was carefully recorded.

Molecular identification of selected bioagents

The isolates bacteria proved to be significant antagonistic against the pathogen were identification by 16 s rRNA sequencing, based on a previous in vitro screening test.

Control of potato soft rot disease by bacterial bioagents

Effect of Brevundimonas bullata, Bacillus siamensis, and Bacillus velezensis on infected potato tubers with P. carotovorum subsp. carotovorum (in vivo)

The pots, each with a diameter of 30 cm, underwent sterilization by immersion in formalin (5%) for 5 min, followed by a week-long period to allow the formalin's poisonous effects to dissipate. They were then filled with autoclaved soil, which had been subjected to autoclaving for 3 h on three consecutive days. For the inoculation of either bioagents or the pathogenic strain of Pcc14, the respective cultures were grown in conical flasks containing 200 ml of autoclaved NSA medium and were then incubated for 48 h at 30 °C. The bacterial bioagents' inoculum was prepared by growing them in flasks containing autoclaved NSA medium, which were subsequently incubated for 2 days at 30 °C (Azaiez et al 2018). Ten potato tubers were washed well with tap water then surface sterilized using 70% ethyl alcohol. Following sterilization, the tubers were soaked for 1 h in a solution containing antagonistic bacteria at a concentration of 2×10^8 CFU/ml. Subsequently, these tubers were allowed to dry overnight in jars. The tubers were then planted in potted soil inoculated with *P. carotovorum* subsp. *carotovorum* (Pcc14). The treatments applied were as follows:

- Control treatments: tubers untreated with pathogen or bioagents planted in sterilized soil.
- Tubers treated individually with antagonistic microorganisms *Brevundimonas bullata*, *Bacillus siamensis*, and *B. velezensis* and then planted in soil inoculated with *P. carotovorum* subsp. *carotovorum* (Pcc14).
- Infected control: Tubers planted directly in soil inoculated with *P. carotovorum* subsp. *carotovorum* (Pcc14).

Each pot was planted with one tuber, and there were five pots per treatment to serve as replicates. These pots were then placed in a greenhouse maintained at a temperature of 28 ± 2 °C and 80% humidity. Careful irrigation was carried out as needed throughout the experiment. At the end of the experiment, the percentages of infection, healthy survivals, and disease severity were recorded based on visual inspection of each tuber, following the method outlined by Chastanger and Ogawa (1979). Infected tubers were categorized into one of five groups based on the severity of infection.

0 = superficial flaking (No Rot) 1 = 1–24% surface decay 2 = 25–49% surface decay 3 = 50–74% surface decay 4 = 75% or more surface decay.

Effectiveness of Brevundimonas bullata, Bacillus siamensis, and Bacillus velezensis in suppressing soft rot disease caused under storage conditions

This experiment aimed to assess the effectiveness of three bacterial bioagents (*B. bullata*, *B. siamensis*, and *B. velezensis*) in suppressing soft rot disease caused Pcc14in potato tubers. The experiment was conducted in two parts: treated tubers and control tubers. In the treated tubers, potato tubers were immersed in a solution containing 50 µg/ml of each plant extract and bacterial bioagents at 10^6 CFU/ml concentrations. The tubers were then allowed to dry completely. Control tubers, on the other hand, were submerged in sterilized distilled water.

After treatment, the tubers were stored at 10 °C for 28 days. Each week, 18 tubers from each treatment group are selected to test their susceptibility to infection with Pcc14. The tubers were inoculated with a 200 µl adjusted suspension (1×10^8 CFU/ml) and incubated at 27–29 °C, while control tubers were inoculated with sterilized distilled water. All treated and control tubers were incubated at the optimum temperature of 27–29 °C for 96 h to observe the development of soft rot according to the method described by Saleh et al. (1996).

Disease severity of the soft rot disease (weight loss) was estimated mathematically using the equation of Yaganza et al. (2004), as follows:

$$\text{Disease Severity Index \% (DSI)} = \frac{\text{Tw1} - \text{Tw2}}{\text{Tw1}} \times 100$$

where Tw_1 = Total weight of tuber. Tw_2 = Total weight of tuber without rotting tissue.

Statistical analysis and experimental design

All treatments here were organized in a complete randomized design. The collected data were analyzed using analysis of variance (ANOVA) with the general linear module procedure outlined by Anonymous (1985). When necessary, treatment means were separated using LSD. Prior to statistical analysis, all percentages were appropriately transformed.

Results

Effect of bioagents on the pathogen

Fifteen isolates were obtained from rhizosphere of potato plant all this isolates were tested against the causal pathogen as in Table 1. Fifteen bacteria isolates were tested in vitro against *P. carotovorum* subsp. *carotovorum*.

Table 1 Effect of certain bacteria isolates in vitro on growth of *Pectobacterium carotovorum subsp. carotovorum*

Isolates (10^8 CFU/ml)	Inhibition zone diameter (cm)
1	2.6 b
2	0.0 g
3	0.0 g
4	0.0 g
5	0.0 g
6	1.0 e
7	0.0 g
8	0.0 g
9	0.0 g
10	1.75 d
11	2.2 c
12	2.0 c
13	0.0 g
14	0.60 f
15	0.0 g
Streptomycin 1.0 mg/ml	2.9 a
Water	0.0 g

Values followed by different letters indicate that means are significantly different from each other according to Fisher's least significant difference (LSD) test at $p \leq 0.05$

From these, six isolates could inhibit the growth of the pathogen. Isolates No. 1, 11, and 12 displayed the highest antagonistic activity (2.2, 2.6, and 2.0 cm, respectively) against Pcc14, while the isolate 14 gave the least inhibition (0.6 cm) of the pathogen growth (Table 1). According to these results, three isolates for the following experiments were selected (isolates No.1, 11, and 12) (Additional file 1: Fig. S1).

Identification of bioagents

The growth reduction of the pathogen was observed with all three bacterial isolates. PCR amplification analysis of these isolates revealed significant similarity to reported strains. Isolate no. 1 strain exhibited 100% similarity to *B. siamensis* (accession no. Nr180225) through BLAST analysis, leading to its identification as *B. siamensis*. The sequence was deposited in NCBI GenBank under accession number PP218278.1. Isolate no. 11 strain showed 98% similarity to *B. velezensis* (accession no. Nr075005) upon BLAST analysis, leading to its identification as *B. velezensis*. The sequence was submitted to NCBI GenBank under accession number PP218280.1. Isolate no. 12 displayed 100% similarity to *B. bullata*. It was submitted to NCBI under accession number PP218279.1. Phylogenetic trees for *B bullata, B. siamensis* and *B. velezensis* strains were constructed (Fig. 1A–C) to elucidate their relationship with other reported species.

Effect of certain *bacteria* bioagents on bacterial soft rot disease severity of potato tubers

The bacteria treatments listed, including *B. bullata*, *B. siamensis*, and *B. velezensis*, demonstrate varying degrees of efficacy in controlling the progression of soft rot disease. Notably, *B. velezensis* showed promising results with relatively low disease severity scores across all time points compared to the other treatments, with values such as no infection at the beginning of the experiment 0 day, 1.4 on day 6, 5 on day 12, 7 on day 18, and 9 on day 28. The infected control group serves as a benchmark, showcasing the natural progression of soft rot disease in the absence of treatment. The significantly higher disease severity scores in the infected control group, reaching values like 15 on day 6, 22 on day 12, 49 on day 18, and 76 on day 28, emphasize the urgency of effective treatment methods to combat this destructive disease. Overall, these findings underscore the potential of certain bacterial treatments, such as *B. velezensis*, in managing soft rot disease and highlight the importance of continued research in this area to develop sustainable agricultural solutions (Fig. 2).

Effect of certain *bacteria* bioagents on bacterial soft rot disease severity of potato tubers under greenhouse conditions

These values depict the disease severity percentage and the corresponding reduction percentage for each bacterial treatment and the infected control group. Notably, *B. velezensis* demonstrated the lowest disease severity percentage at 7.00% and the highest reduction percentage at 86.00%, indicating its efficacy in mitigating soft rot disease compared to the other treatments. *B. bullata* shows a disease incidence of 6.00%, indicating a moderate level of occurrence of soft rot disease. Treatment with *B. bullata* results in a reduction of disease incidence by 70.00%, suggesting a significant effectiveness in reducing the occurrence of the disease compared to the infected control group. *B. siamensis* exhibits a disease incidence of 8.00%, slightly higher than *B. bullata*. The reduction percentage of 60.00% indicated a moderate effectiveness in reducing disease incidence. *B. velezensis* demonstrated the lowest disease incidence percentage at 2.00%, signifying effective control of soft rot disease occurrence. Additionally, the reduction percentage of 90.00% highlights its strong efficacy in reducing disease incidence compared to the other treatments. These values underscore the effectiveness of *B. velezensis* in significantly reducing the

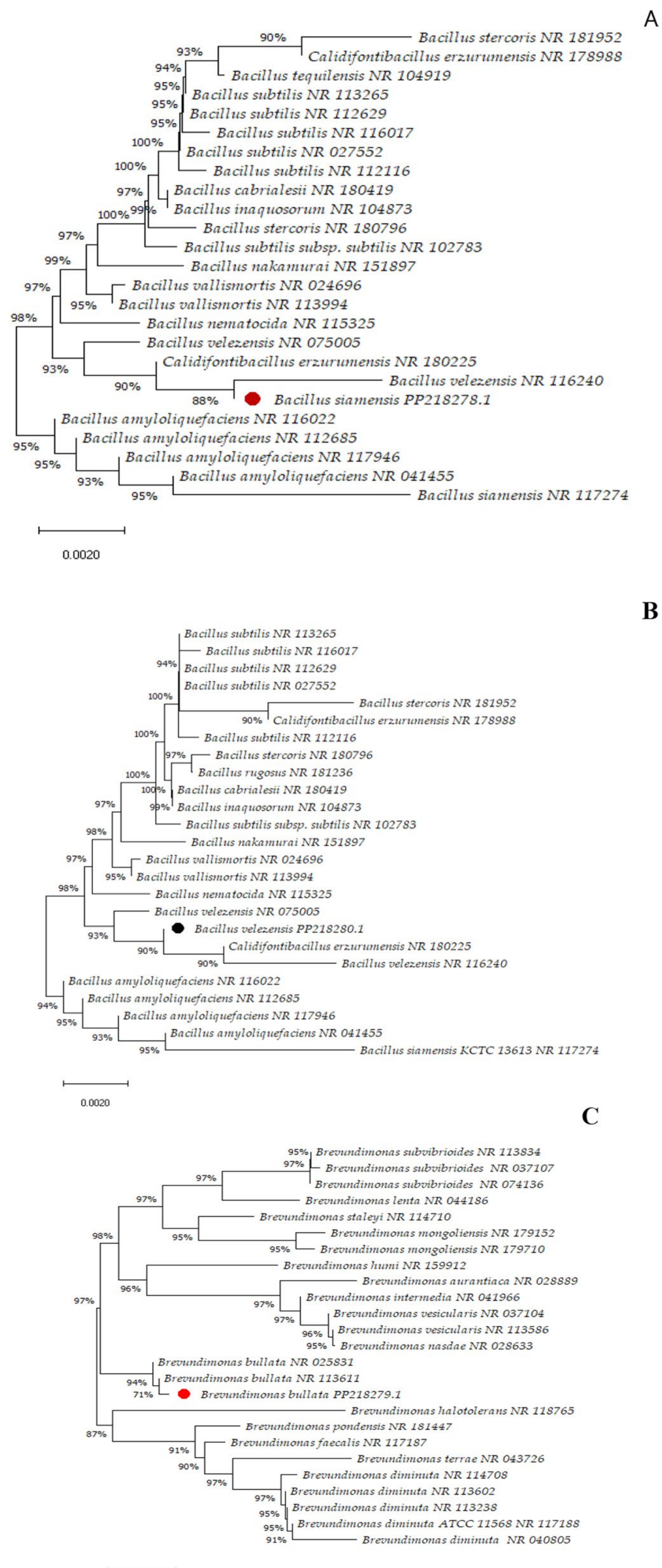

Fig. 1 Semistrict concurrence of most-parsimonious trees based on retrieved bacteria sequences data from NCBI public domain **A** *Bacillus siamensis*, **B** *Bacillus velezensis*, and **C** *Brevundimonas bullata*. This tree was compatible overall in highly supported lineages to the Bayesian 50% majority-rule consensus tree. Jackknife frequencies (10,000 replicates) are shown above each node. Red color indicates the relationship of identified bacteria isolate of the present study.

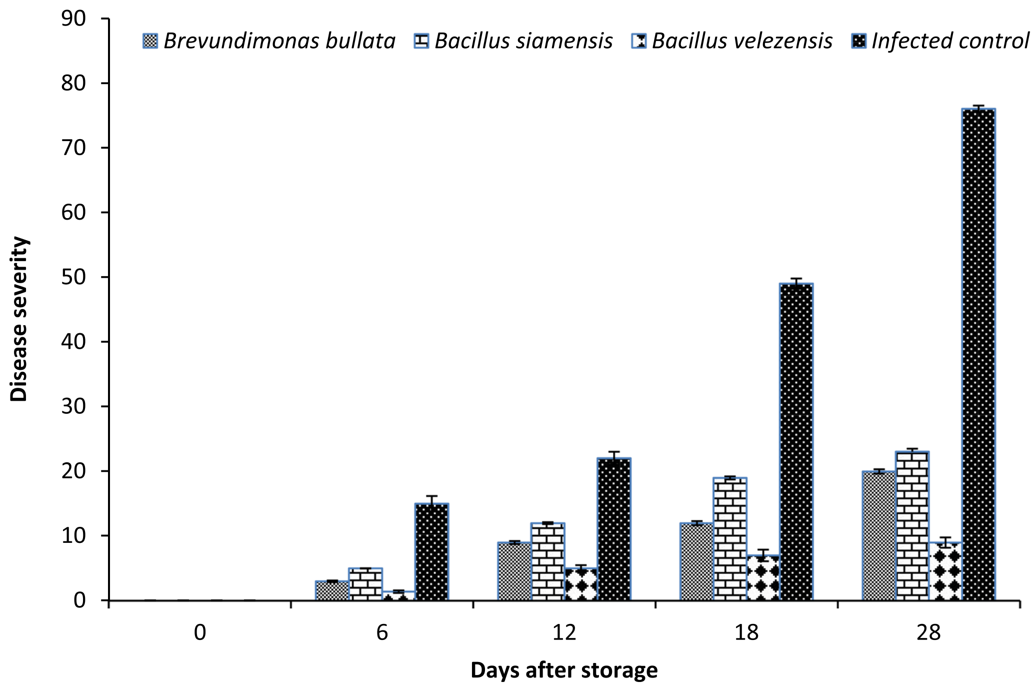

Fig. 2 Effect of Bacillus *siamensis, Bacillus velezensis*, and *Brevundimonas bullata* on bacterial soft rot disease severity of potato tubers during 28 days storage. Error bars on each graphs represent the Mean ± SE.

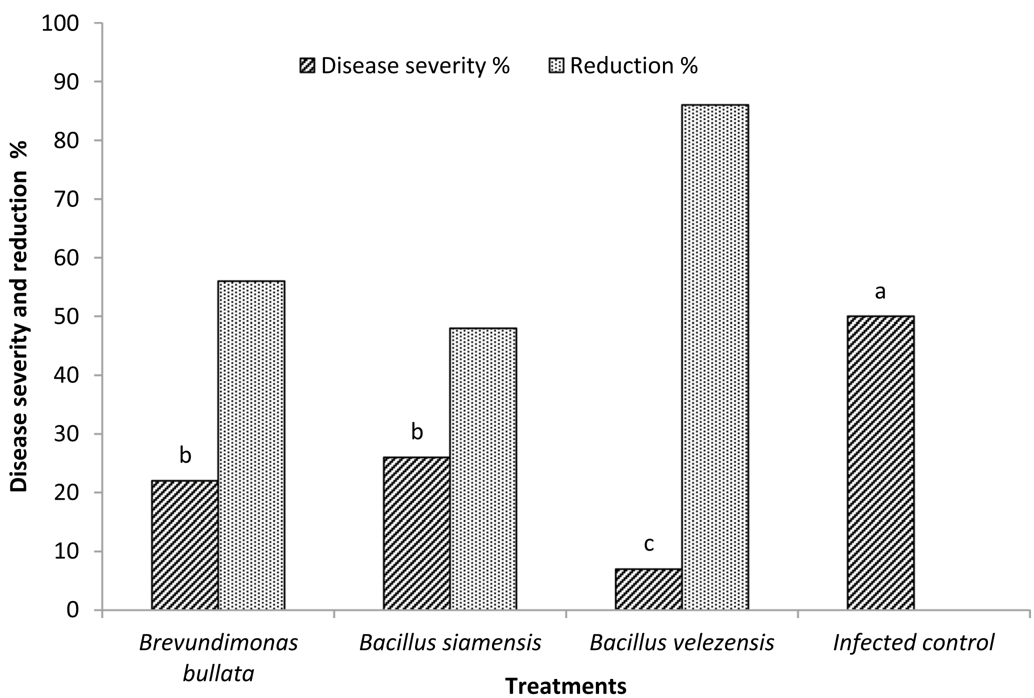

Fig. 3 Effect of *Bacillus siamensis, Bacillus velezensis*, and *Brevundimonas bullata* on bacterial soft rot disease severity of potato tubers under greenhouse conditions. Values followed by different letters indicate that means are significantly different from each other according to Fisher's least significant difference (LSD) test at $p \leq 0.05$.

occurrence of soft rot disease, indicating its potential as an effective treatment option in agricultural settings (Fig. 3).

Discussion

In crop production, tackling bacterial diseases has emerged as a significant concern recently, primarily because of the scarcity of viable bactericides that are both effective and approved for use. Traditionally, copper-based bactericides and antibiotics like streptomycin have been relied upon for managing plant bacterial infections, leading to the emergence of resistant strains over time. Consequently, there is an urgent need to devise a sustainable and potent control approach to address the growing threat posed by these resilient bacterial pathogens (Abdelghany et al 2022).

More recently, disease management has seen advancements through adopting disease-resistant cultivars and utilizing targeted bacteriophages and antimicrobial peptides synthesized by microbes. These innovative approaches have proven effective in dampening the virulence of pathogenic bacteria, offering promising alternatives in the ongoing battle against agricultural diseases Ashmawy et al. (2015).

The present research has revealed the efficacy of soil-derived *Brevundimonas bullata, Bacillus siamensis*, and *B. velezensis* in combating *P. carotovorum*, the pathogen responsible for soft rot. In vitro experiments demonstrated that all tested bacteria could impede the growth of *P. carotovorum* on solid media. Notably, these bioagents' inhibition of *P. carotovorum* growth was evidenced by forming a clear zone on a solid medium within two days post-inoculation.

Several studies have highlighted *Bacillus* species as potent sources of various antimicrobial compounds. Zhao et al. (2013) also observed that *Bacillus amyloliquefaciens* inflicted severe damage on potato wounds. However, when potato tubers were treated with the cell-free supernatant of this antagonistic strain, significant protection was achieved, indicating its potential for disease control. Algeblawi and Adam (2013) documented the effectiveness of bioagents like *Pseudomonas fluorescens, Bacillus subtilis*, and *Bacillus thuringiensis* in mitigating soft rot disease in potato tubers caused by *E. carotovora* subsp. *carotovora* in pot experiments. Particularly promising outcomes were observed with isolates of *P. fluorescens* and *B. subtilis*, outperforming the control treatment.

In the present study, in vitro tests of biocontrol agents showed varying degrees of antagonistic effects against *P. carotovorum* subsp. *carotovorum* isolates. *B. velezensis* demonstrated stronger antagonistic effects against soft rot bacteria than *B. bullata* and *B. siamensis*. These findings align with those reported by Ryan et al. (2001), who observed that *B. subtilis* and *B. amyloliquefaciens* could indirectly enhance plant growth by inducing systemic resistance (ISR). This occurs through the secretion of volatiles, which activate an ISR pathway in Arabidopsis seedlings challenged with the soft rot pathogen *E. carotovora* subsp. *carotovora* (Rashid et al. 2013).

The storage findings revealed that the tested biocontrol agents shielded whole potato tubers against soft rot disease. *B. velezensis* exhibited the highest level of protection, followed by *B. bullata* and *B. siamensis*. These results are consistent with those of Rahman et al. (2012) reported, who noted that *Bacillus* species demonstrated significant antagonistic effects against *E. carotovora* subsp. *carotovora* in both in vitro tests and up to 22 weeks of potato storage. Additionally, Makhlouf and Abdeen (2014) found that combining *B. subtilis, P. fluorescens*, displayed potent antagonistic activity against the growth of *E. carotovora* subsp. *carotovora* in vitro and during storage.

Conclusions

The bacterial biocontrol agents such as *B. bullata*, *B. siamensis*, and *B. velezensis* demonstrated significant efficacy in controlling various isolates of Pcc, responsible for soft rot disease, as evidenced by both in vitro and in vivo tests. The results highlighted variability in the antagonistic activity of the tested biocontrol agents against bacterial soft rot isolates in vitro. However, in in vivo tests, *B. velezensis* was highly effective in reducing soft rot symptoms on inoculated potato tuber slices. Moreover, in the whole potato tubers technique, *B. velezensis* provided superior protection to *B. bullata* and *B. siamensis*, respectively. These findings underscore the potential of *B. velezensis* as a valuable tool in controlling bacterial soft rot disease in vegetables, suggesting its practical application in agricultural settings. Overall, this study underscores the importance of addressing soft rot in potato production, as it adversely affects the quality and yield of this vital crop overall world. (Additional file 1: Fig. S1).

Abbreviations

Pcc	*P. carotovorum* Subsp. *carotovorum*
NSB	Nutrient sucrose agar
NSB	Nutrient sucrose broth
KSA	Kingdom of Saudi Arabia
SD	Standard deviation
LSD	Least significant difference
cfu/ml	Colony-forming unit

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-024-00794-4.

Additional file 1: Fig. 1S. Effect of certain bacteria isolates in vitro on growth of Pectobacterium carotovorum subsp. carotovorum.

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Author contributions
Abo-Elyousr and Almasoudi contributed to conceptualization, methodology, formal analysis, and writing—original draft, Al-Qurashi was involved in supervision and review and editing, Mohamed I. Elsayed contributed to conceptualization and formal analysis, and Abo-Elyousr was involved in review and editing, formal analysis, and review and editing.

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Not applicable. This manuscript is in accordance with the guide for authors available on the journal's website. Also, this work has not been published previously and is approved by all authors and host authorities.

Consent for publication
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Competing interests
No potential conflict of interest was reported by the authors.

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