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Antifungal activity of *Bacillus amyloliquefaciens* filtrate against *Fusarium* dry rot of potato

Antifungalna aktivnost filtrata *Bacillus amyloliquefaciens* protiv fuzarijske suhe truleži krumpira

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ABSTRACT

Potato (*Solanum tuberosum*) tuber dry rot caused by *Fusarium* spp. leads to substantial losses during storage and may be exacerbated by secondary soft-rot bacteria. Due to limitations of chemical control, *Bacillus*-based biocontrol is increasingly explored as a sustainable alternative. This study evaluated the antagonistic potential of *Bacillus amyloliquefaciens* culture filtrate against *Fusarium oxysporum* and *F. graminearum*. *In vitro* poisoned food assay with 1% (v/v) filtrate showed strong inhibition of mycelial growth, reaching 93% for *F. oxysporum* and 91% for *F. graminearum* after 7 days compared to the control. *In vivo* preventive treatment of wounded potato tubers significantly reduced dry rot development after 21 days, by 72% (*F. oxysporum*) and 84% (*F. graminearum*). Microscopic observations revealed pronounced hyphal abnormalities in treated variants (hyphal swelling, vacuolisation and increased chlamydospore formation), indicating severe pathogen stress. Overall, the results demonstrate that *B. amyloliquefaciens* filtrate is a highly effective biocontrol agent against potato *Fusarium* dry rot, with potential for inclusion in sustainable postharvest disease management.

Keywords: potato, *Bacillus amyloliquefaciens*, *Fusarium oxysporum*, *Fusarium graminearum*, biological control

SAŽETAK

Suha trulež gomolja krumpira uzrokovana vrstama roda *Fusarium* jedan je od najznačajnijih skladišnih problema te se može dodatno pogoršati sekundarnim bakterijskim infekcijama koje uzrokuju vlažnu trulež. Zbog ograničene učinkovitosti kemijskog suzbijanja, biološko suzbijanje temeljeno na bakterijama roda *Bacillus* sve je važnija održiva alternativa. U ovom radu, procijenjen je antagonistički potencijal filtrata kulture *Bacillus amyloliquefaciens* u suzbijanju patogena *Fusarium oxysporum* i *F. graminearum*. Tretman proveden metodom poisoned food u uvjetima *in vitro* s 1 % (v/v) filtrata pokazao je snažnu inhibiciju micelarnog rasta nakon 7 dana i to od 93 % kod *F. oxysporum* te oko 91 % kod *F. graminearum* u odnosu na kontrolu. Preventivni tretman *in vivo* ranjenih gomolja krumpira filtratom značajno je smanjio razvoj suhe truleži nakon 21 dana za 72 % (*F. oxysporum*) i 84 % (*F. graminearum*). Mikroskopska analiza ukazala je na izražene abnormalnosti hifa u tretmanima (oticanje i povećana tvorba hlamidospora), što upućuje na snažan stres patogena. Rezultati potvrđuju da je filtrat *B. amyloliquefaciens* vrlo učinkovit biološki čimbenik s potencijalom primjene u održivom suzbijanju fuzarioza gomolja krumpira.

Ključne riječi: krumpir, *Bacillus amyloliquefaciens*, *Fusarium oxysporum*, *Fusarium graminearum*, biološko suzbijanje

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide and belongs to the family *Solanaceae*, which comprises a large number of cultivated species and varieties. Due to its high nutritional value and broad use in human nutrition, the potato represents a key food source in many parts of the world, with an estimated annual production of approximately 375 million tonnes (Rondon et al., 2022; FAOSTAT, 2022). The crop also holds considerable national importance, as Croatia produces more than 126,000 tonnes of potatoes per year, underscoring the economic relevance of effective disease management strategies (DZS, 2024). However, potato yields are continually challenged by multiple abiotic and biotic stress factors, including extreme climatic conditions, improper handling during and after harvest, as well as numerous diseases affecting both foliage and tubers (Tiwari et al., 2021; Hussain et al., 2022). To date, more than 40 economically important potato diseases have been documented, including those caused by bacteria, viruses, fungi, nematodes and insects, all of which contribute to substantial losses in the field and during storage. Among the most destructive pathogens is the fungal genus *Fusarium*, particularly species such as *Fusarium oxysporum* (Schlecht. emend. Snyder and Hansen) and *Fusarium graminearum* (Schwabe), which cause potato dry rot, one of the most widespread and economically significant storage diseases (Tiwari et al., 2021). This disease can result in losses ranging from 25% to 60%, leading to tuber tissue decay, root system degeneration and, in severe cases, plant wilting in the field (Bojanowski et al., 2013; Heltoft et al., 2016). Infections commonly develop at wound sites created during harvesting or transport, where fungal spores can penetrate the tissue (Xue and Yang, 2021). An additional concern is the ability of *Fusarium* spp. to produce mycotoxins, which not only reduce tuber quality but also pose serious health risks to humans and animals (Chen et al., 2020). Potato dry rot caused by *Fusarium* spp. may become even more destructive when secondary bacterial infections develop, leading to soft or wet rot. Bacteria, primarily species of the genus *Pectobacterium*, frequently colonise dry rot lesions, especially under conditions of high humidity, resulting in wet, slimy decay that can mask initial symptoms and rapidly accelerate tuber breakdown (Bojanowski et al., 2013). Traditional disease management strategies rely heavily on synthetic fungicides, whose efficacy varies and whose repeated use can lead to phytotoxic residues and disruption of soil ecological balance (Fothergill and Abdelghani, 2013). In recent years, increasing attention has been directed towards developing sustainable biological control strategies that include the application of beneficial microorganisms, natural compounds and environmentally friendly technologies (Sofi et al., 2021; Hussain et al., 2022; Lahlali et al., 2022). Among these, *Bacillus* species stand out due to their ability to produce a wide range of bioactive metabolites with antifungal properties, to induce plant resistance and to inhibit pathogen development both in the field and during storage (Shafi et al., 2017; Lastochkina et al., 2020). Lipopeptides and other antimicrobial compounds synthesised by these bacteria have been recognised as key factors underlying their effectiveness against *Fusarium* spp. (Upadhyay and Jayaswal, 1992; Ongena and Jacques, 2008). Given the long-term persistence of these pathogens in soil, their detrimental effects on yield and tuber quality, and the limited success of conventional control measures, there is a clear need to develop more effective and sustainable approaches for managing potato dry rot (Panth et al., 2020). Biological agents, including *Bacillus*-based formulations, are increasingly recognised as promising alternatives within integrated disease management strategies, making this line of research particularly relevant (Basco et al., 2017; Shafique et al., 2017). According to the Croatian Phytosanitary Information System, no registered products for managing potato tuber dry rot are currently available on the national market, including biocontrol agents based on *Bacillus* species (FIS, 2025), which further emphasises the urgent need for effective biological alternatives.

MATERIALS AND METHODS

Cultivation of Bacillus amyloliquefaciens

The strain *B. amyloliquefaciens* was isolated from the commercial biocontrol product Taegro (Syngenta) and cultured in 100 mL of liquid Potato Dextrose Broth (PDB) (Sigma-Aldrich, Darmstadt, Germany) at 28 °C for 48 h to prepare the bacterial inoculum. The liquid culture was prepared according to the procedure described by Khedher et al. (2021). After incubation, the culture was centrifuged at 6000 × g for 10 min; the supernatant was removed, and the bacterial cell pellet was resuspended in sterile distilled water and adjusted to a final concentration of 10^8 cells/mL.

Cultivation of phytopathogenic fungi

Two fungal isolates were used in this study: *F. graminearum* and *F. oxysporum*, morphologically identified according to Leslie and Summerell (2006). Both isolates were originally obtained from potato tubers showing dry rot symptoms, purified, and cultured on Potato Dextrose Agar (PDA) (Sigma Aldrich, Darmstadt, Germany) followed by incubation at 25 °C in darkness.

Potato tubers

Potato tubers (*S. tuberosum*) of the cultivar 'Spunta' were used for the experiments. Tubers were previously stored at 6 °C for one month. Immediately prior to experimentation, they were surface-sterilised by immersion in a 10% sodium hypochlorite solution for 5 min, rinsed with sterile distilled water, and air-dried.

In vitro evaluation of the antifungal activity of Bacillus amyloliquefaciens

The antagonistic effect of *B. amyloliquefaciens* filtrate against *F. oxysporum* and *F. graminearum* was tested following the method of Khedher et al. (2021). The filtrat was obtained by centrifuging the bacterial culture and passing it through a sterile membrane filter (0.45 µm). It was incorporated into molten PDA at 1% v/v. After the medium solidified, a 5 mm mycelial plug from a 7-day-old fungal culture was placed in the center of each Petri dish. Plates were incubated at 25 °C in darkness for 7 days. Control plates were prepared identically but supplemented with an equal volume of PDB instead of the bacterial supernatant. Each treatment included five replicates per fungal species.

In vivo evaluation of the Bacillus amyloliquefaciens antifungal activity on potato tubers

The inhibitory activity of *B. amyloliquefaciens* was further evaluated *in vivo* on potato tubers. Surface-sterilised tubers were wounded using a sterile cork borer to create three cavities (5 mm diameter, 5 mm depth) per tuber. Each wound was treated with 50 µL of bacterial supernatant (10^8 cells/mL), and, after one hour, inoculated with 50 µL of a fungal conidial suspension (10^7 conidia/mL). Control tubers received only the pathogen suspension. Wounds were sealed with parafilm, and tubers were incubated at 25 °C in darkness for 21 days. The experiment was arranged in two variants per pathogen, with six replicates (three tubers × two wounds) according to a modified method of Khedher et al. (2021).

Data collection and statistical analysis

The mycelial growth area on PDA plates and the lesion area on tuber slices were quantified by photographing samples alongside a measurement scale from a 50 cm distance, ensuring the camera lens was positioned perpendicular to the surface. The mycelial growth area (cm^2) of each *Fusarium* isolate was measured, and mean values were used to calculate the inhibition index (%). This index served to quantify the antifungal activity of *B. amyloliquefaciens* against *F. oxysporum* and *F. graminearum*. Images of test and control plates and tuber slices were analysed using the ImageJ software following Martinko et al. (2022). Statistical comparisons between treatments and controls were performed using Student's t-test in SPSS. Antifungal activity was further confirmed through microscopic examination of fungal morphological changes using a light microscope (BH2, Olympus, Japan).

RESULTS AND DISCUSSION

The results of this study clearly demonstrate the strong antifungal potential of *B. amyloliquefaciens* filtrate against the two major causal agents of potato dry rot, *F. oxysporum* and *F. graminearum*. Under *in vitro* conditions, the application of 1% filtrate resulted in 93% inhibition of *F. oxysporum* and approximately 91% inhibition of *F. graminearum* which was statistically significant according to the Student's t-test ($P < 0.05$) compared with the control (Figure 1).

This magnitude of mycelial growth suppression substantially exceeds the values reported in previous studies, where Khedher et al. (2021) recorded 55% inhibition for *F. oxysporum* and 74% for *F. graminearum*, while Daami Remadi et al. (2006) reported inhibition levels of 62–64% for *F. oxysporum*. These differences indicate that the tested *B. amyloliquefaciens* strain, as well as its metabolites present in the filtrate, exhibit exceptionally strong antifungal activity even at low application concentrations.

A similar trend was observed under *in vivo* conditions. Preventive treatment of potato tubers with the filtrate reduced rot development caused by *F. oxysporum* by 72%, while rot caused by *F. graminearum* was reduced by approximately 84% (Figure 2). While treated tubers exhibited markedly reduced lesion areas, control tubers frequently developed extensive soft, wet decay. In several control samples, the initially dry *Fusarium* lesions progressed into wet, macerated rot indicative of secondary bacterial infection, a phenomenon commonly associated with opportunistic bacteria such as *Pectobacterium* spp., which readily colonise *Fusarium*-damaged tissues (Bojanowski et al., 2013).

Such combined *Fusarium*-bacterial infections are well-documented and contribute to rapid tissue breakdown and accelerated deterioration of potato tubers during storage. The clear contrast between the extensive wet decay in the control and the substantially reduced rot in treated tubers highlights the protective effect of *B. amyloliquefaciens*.

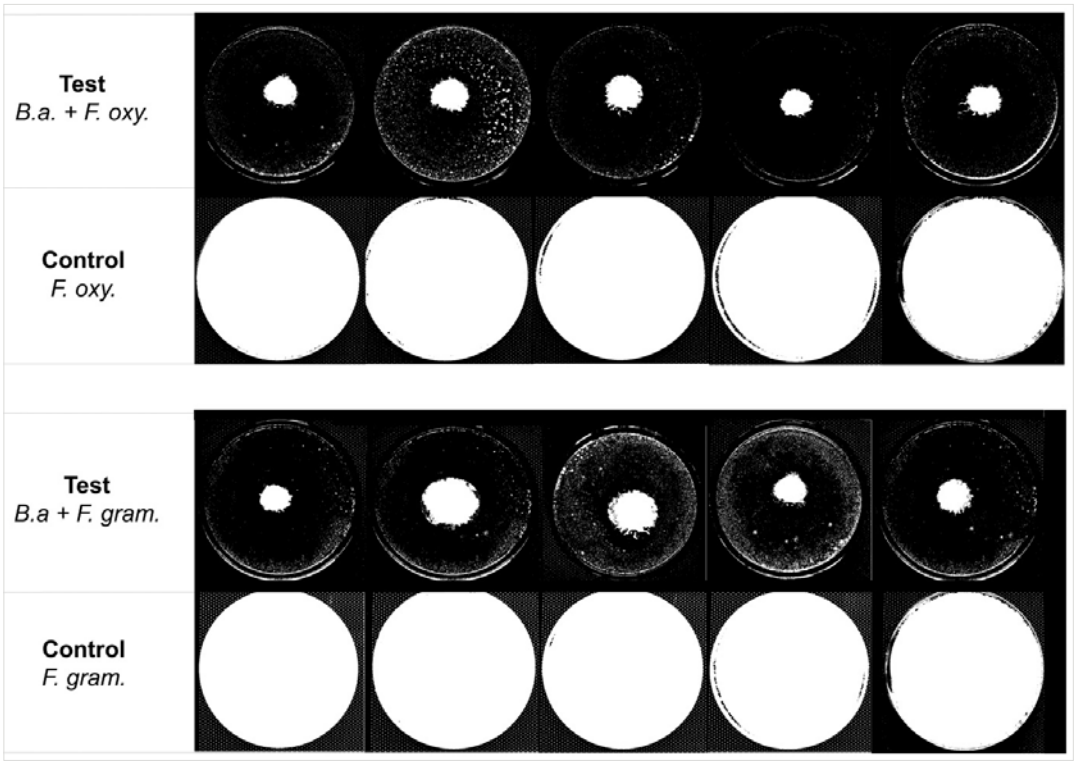


Figure 1. Antifungal effect of 1% *Bacillus amyloliquefaciens (B.a.)* filtrate on the mycelial growth of *Fusarium oxysporum (F. oxy.)* and *Fusarium graminearum (F. gram.)* after 7 days in comparison with the control (based on ImageJ image analysis in which the white area represents fungal mycelial growth)

Notably, *F. graminearum* was about 12% more sensitive to the filtrate, which may suggest a stronger physiological response to *Bacillus*-derived metabolites or a reduced ability to develop adaptive mechanisms under high biological stress. Such pathogen-specific differences are rarely addressed in the literature, emphasising the importance of this finding. For comparison, Khedher et al. (2021) reported 54% reduction of *F. oxysporum* rot and a 43% reduction of *F. graminearum* rot, demonstrating that *B. amyloliquefaciens* in the present study exhibited superior protective efficacy. The high effectiveness of the genus *Bacillus* has been confirmed in numerous studies. Wharton and Kirk (2007) documented reductions in dry rot incidence on seed tubers following treatments

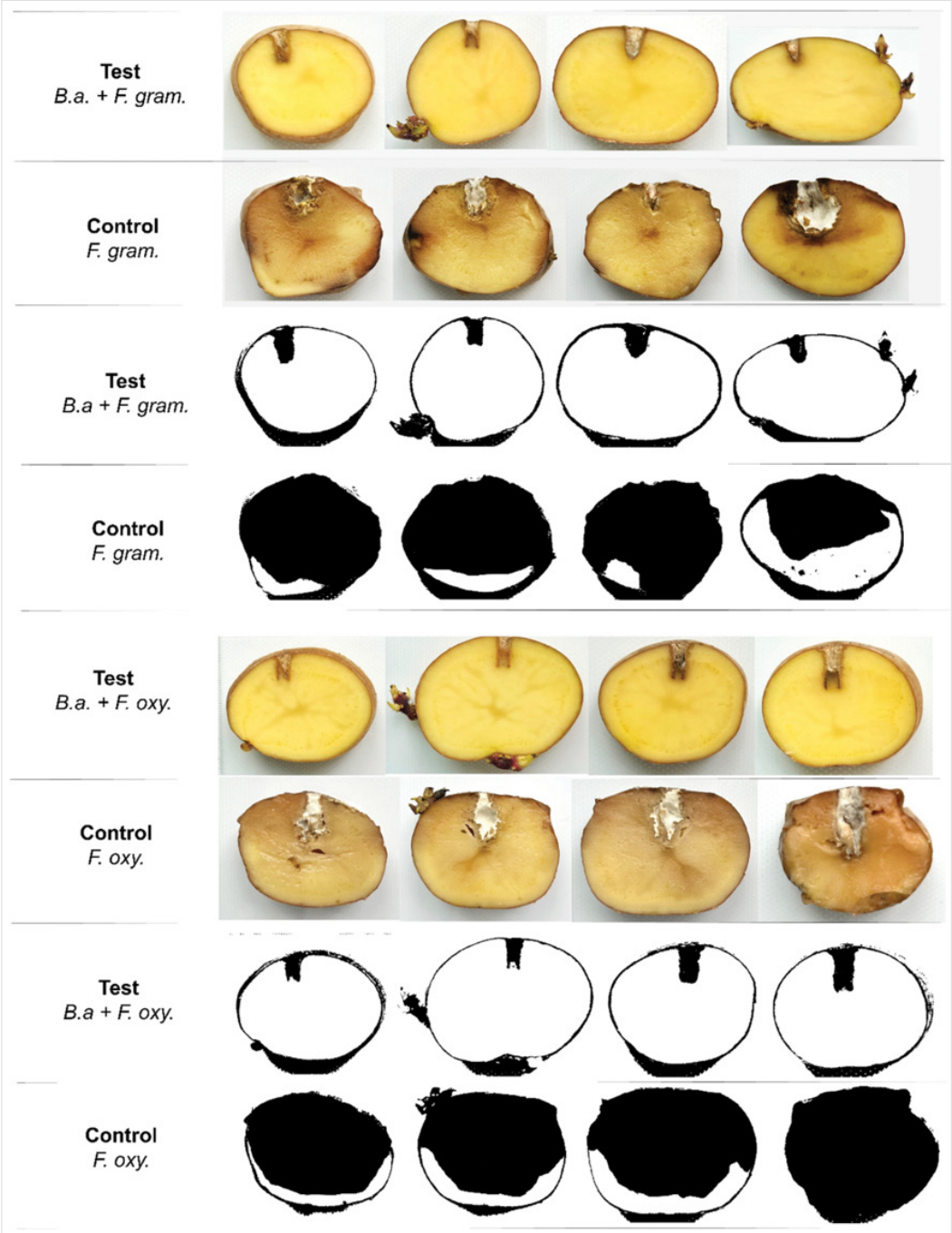


Figure 2. Antifungal effect of 1% *Bacillus amyloliquefaciens (B.a.)* filtrate on potato tubers inoculated with *Fusarium oxysporum (F. oxy.)* and *Fusarium graminearum (F. gram.)* after 21 days, in comparison with the control (based on ImageJ image analysis in which the black area represents the extent of tuber rot)

with *Bacillus*-based bioproducts, while Al-Mughrabi et al. (2008) reported reduced rot development and morphological deformation of *Fusarium* hyphae following treatment with a *Bacillus* isolate. Khedher et al. (2021) recorded 42.8–63.8% reductions in dry rot severity, and Lastochkina et al. (2020) demonstrated significant suppression of *F. oxysporum* during storage following treatment with *Bacillus subtilis*. Additionally, Hao et al. (2024) reported 68–76% inhibition of *F. oxysporum* by *B. subtilis* filtrate, while *Bacillus firmus*-based formulations have been shown to inhibit *Rhizoctonia solani* by nearly 88% and reduce potato disease incidence by 71% in laboratory conditions and 45% under field conditions (Hussain et al., 2022).

Antifungal explanations for these effects are strongly supported by the morphological observations in the present study. Microscopic analysis revealed that hyphae of both pathogens exposed to the 1% filtrate exhibited weak, sparse and morphologically altered mycelium, with clear abnormalities such as hyphal swelling, vacuolisation and intensified formation of chlamydospores (Figure 3a). In contrast, control samples developed dense, well-branched and homogeneous mycelium with intact, turgid hyphae (Figure 3b), confirming that the observed deformities were a direct effect of the bacterial filtrate.

Such hyphal deformities are typical indicators of severe metabolic stress induced by biological suppression. According to De Avila Santos et al. (2022), *Bacillus* spp. synthesise organic acids and other bioactive compounds that destabilise fungal membranes, impair osmoregulation and disrupt metabolic pathways essential for growth. Gwon et al. (2015) similarly reported vacuolisation, cell wall damage and enhanced chlamydospore formation in fungi exposed to *Bacillus* filtrates. These observations align fully with the findings of this study, indicating that the pathogen activated survival mechanisms following exposure to the filtrate.

Given the exceptionally high inhibition rates observed both *in vitro* and *in vivo* in this study, the tested *B. amyloliquefaciens* likely produces a broad spectrum of bioactive metabolites (including lipopeptides, enzymes and organic acids) which are well-documented contributors to antifungal efficacy in *Bacillus* spp. (Upadhyay and Jayaswal, 1992; Ongena and Jacques, 2008; De Avila Santos et al., 2022). Detailed chemical profiling of these compounds would therefore be valuable for future investigations. Overall, the results of this study (encompassing strong inhibition of mycelial growth, substantial suppression of tuber rot, and pronounced hyphal deformities) are fully consistent with previous research

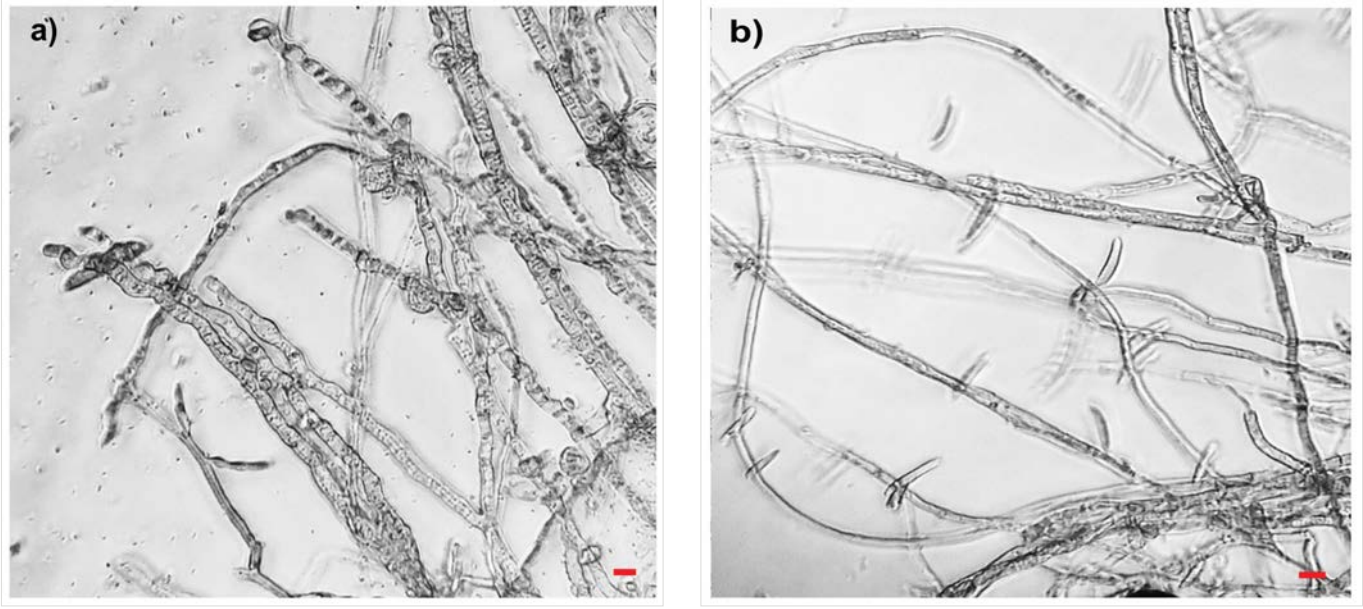


Figure 3. Microscopic view of *Fusarium oxysporum* hyphae grown on PDA amended with 1% *Bacillus amyloliquefaciens* filtrate (a) compared with control hyphae (b) after 7 days (scale bar: 10 µm)

demonstrating the effectiveness of *Bacillus* species as biocontrol agents against *Fusarium* pathogens (Wharton and Kirk, 2007; Al-Mughrabi et al., 2008; Khedher et al., 2021; Hussain et al., 2022; Hao et al., 2024). The stability, efficacy and environmental safety of *Bacillus amyloliquefaciens* highlight its potential as one of the most promising candidates for integration into sustainable plant protection strategies, particularly in the context of reducing reliance on synthetic fungicides in postharvest disease management (Panth et al., 2020; Lahlali et al., 2022).

CONCLUSION

The results of this study demonstrate that *B. amyloliquefaciens* possesses a remarkably strong antagonistic capacity against two major causal agents of potato dry rot, *F. oxysporum* and *F. graminearum*. The 1% bacterial filtrate achieved exceptionally high levels of mycelial inhibition *in vitro* (93% and 91%, respectively) and significantly reduced disease severity *in vivo* (72% and 84%, respectively). Microscopic observations further confirmed substantial structural damage to fungal hyphae, including swelling and enhanced chlamydospore formation, indicating severe physiological stress induced by bacterial metabolites. These findings are consistent with previous research and highlight the broad-spectrum antifungal activity of *Bacillus* sp. Considering the absence of registered chemical or biological products for dry rot management in Croatia, as reported by the Phytosanitary Information System, *B. amyloliquefaciens* emerges as a promising and environmentally sustainable alternative for integrated potato disease management. Overall, the study confirms that *B. amyloliquefaciens* has high potential as a biocontrol agent suitable for postharvest protection and for reducing dependence on synthetic fungicides. Further research, particularly focused on metabolite profiling, formulation development and field validation, is recommended to support commercial application and maximise its effectiveness under practical storage and production conditions.

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