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Māris Seņkovs * , Vizma Nikolajeva , Luīze Rubene , Kristians Jauga , Līga Zemeca , Inta Jakobija

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Antifungal Potential of *Bacillus*, *Streptomyces* spp. and *Trichoderma asperellum* Against Phytopathogenic Fungi

Māris Seņkovs [1,*], Vizma Nikolajeva [1], Luīze Rubene [1], Kristians Jauga [1], Līga Zemeca [2] and Inta Jakobija [2]

[1] Microbial Strain Collection of Latvia, MIRRI-ERIC consortium, Faculty of Medicine and Life Sciences, University of Latvia, Jelgavas street 1, Riga, LV-1004, Latvia

[2] Institute of Plant Protection Research "Agrihorts", Latvia University of Life Sciences and Technologies, Paula Lejiņa street 2, Jelgava, LV-3004, Latvia

* Correspondence: maris.senkovs@lu.lv

Abstract

The increasing demand for sustainable plant protection products has intensified interest in microbial biocontrol agents (BCAs). The antifungal activity of ten actinobacterial strains of the genus *Streptomyces* were evaluated against phytopathogenic fungi *Botrytis cinerea*, *Colletotrichum salicis*, *Fusarium oxysporum* and *F. graminearum* using the dual-culture assay. All tested *Streptomyces* isolates exhibited antifungal activity, with *S. venezuelae* MSCL 350 demonstrating the strongest inhibition of fungal growth. The antifungal activity of *T. asperellum* MSCL 309, *Bacillus subtilis* MSCL 49, *B. subtilis* MSCL 1441 and *B. stercoris* MSCL 897 against twelve *Fusarium* spp. isolates obtained from oats was evaluated. *T. asperellum* effectively inhibited *F. sporotrichioides*, *F. oxysporum*, *F. culmorum* and *F. poae* in dual culture assay while minimal inhibition was observed against the *F. graminearum* strains used. Soluble metabolites produced by *T. asperellum* showed strong antifungal activity against *Fusarium* spp., whereas no significant inhibitory effect of volatile compounds was detected. *B. subtilis* MSCL 1441 inhibited the growth of all tested *Fusarium* isolates, while other two *Bacillus* strains showed no detectable antifungal activity. The results confirm the significant antifungal potential of selected *Streptomyces*, *Trichoderma*, and *Bacillus* strains and support their prospective application as environmentally friendly BCAs against phytopathogenic fungi.

Keywords: *Fusarium; Streptomyces; Bacillus; Trichoderma asperellum; Colletotrichum salicis;* antifungal

1. Introduction

Phytopathogenic fungi represent one of the major constraints to global crop production, causing significant yield losses and compromising food safety. Among them, the genus *Fusarium* is particularly important due to its wide distribution in soils and plant residues and its ability to infect a broad range of agricultural crops. Species such as *Fusarium oxysporum* and *F. solani* are responsible for vascular wilt, root rot, and seedling diseases in cereals, vegetables, and fruit crops [1,2]. *F. graminearum* is a causal agent of *Fusarium* head blight (FHB) of wheat, barley and other small-grain cereals worldwide [3], however other species are also involved. Over the past two decades, the most common species causing FHB in Canada were *F. avenaceum, F. equiseti, F. graminearum, F. poae,* and *F. sporotrichioides* [4]. *F. avenaceum, F. culmorum, F. graminearum, F. poae* and *F. sporotrichioides* have been identified in Latvian populations of *Fusarium* species that cause FHB in both spring barley and oat [5]. *Fusarium* pathogenicity is associated with the secretion of cell wall–degrading enzymes, toxins, and effector molecules that facilitate host penetration, tissue maceration, and systemic colonization [6,7]. In addition to direct plant damage, many *Fusarium* species produce mycotoxins—including trichothecenes, zearalenone, and fumonisins—that threaten animal and human health [8,9].

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F. graminearum and *F. oxysporum* along with *Botrytis cinerea* and *Colletotrichum* spp. are named among the "top 10" based on their scientific/economic importance [10]. WHO also included *Fusarium* species in the group of high-priority pathogens among fungi that can cause invasive acute and subacute systemic fungal infections in humans, and they may also have inherent resistance to many currently available antifungal agents [11].

B. cinerea, a necrotrophic phytopathogenic fungus, the causal agent of grey mould, has a wide host range, and this fungus causes damage both pre-harvest and during crop storage [12]. *Colletotrichum* species, hemibiotrophic fungi, cause anthracnose spots and blights in a wide range of woody and herbaceous plants [13]. *C. salicis* belongs to *C. acutatum* species complex and is associated with anthracnose of woody hosts [14] and fruit rot in peppers and tomatoes [15].

Growing environmental and regulatory pressure to reduce synthetic fungicide use has intensified interest in biological control strategies. Soil microorganisms with antifungal properties are increasingly considered sustainable alternatives in integrated plant protection systems. Among the most extensively studied and promising biocontrol agents (BCAs) are *Trichoderma, Bacillus,* and *Streptomyces* species [16].

Trichoderma species are well-documented antagonists of *Fusarium* spp. and other soil-borne pathogens. Their biocontrol activity is based on three principal mechanisms: mycoparasitism, antibiosis, and competition for nutrients and ecological niches [17,18]. During mycoparasitism, *Trichoderma* attaches to pathogen hyphae, forms appressorium-like structures, and secretes hydrolytic enzymes such as chitinases and β-1,3-glucanases, leading to cell wall degradation and growth inhibition [19,20]. In addition, *T. asperellum* produces secondary metabolites with antifungal activity and can induce systemic resistance in plants, thereby enhancing host tolerance to infection [21]. Commercially available products for agriculture contain various *Trichoderma* species, including strains of *T. asperellum* [22].

Members of the genus *Bacillus* are Gram-positive, endospore-forming bacteria widely distributed in soil environments and known for their stability and adaptability [23]. Their antifungal activity is mainly due to the production of various antifungal volatile and water-soluble compounds, including cyclic lipopeptides such as iturin, surfactin, and fengycin, which disrupt fungal cell membranes and cause leakage of cell contents [24,25]. Additional mechanisms include secretion of lytic enzymes, siderophore-mediated iron competition, and induction of plant defense-related genes [26,27]. Experimental studies have demonstrated significant inhibition of *Fusarium* growth and sporulation by various *Bacillus* strains [28]. *B. subtilis* is a model organism and the most popular workhorse in the biotechnology industry. [29] classified the bioactive metabolites produced by *B. subtilis* into five classes: non-ribosomal peptides, polyketides, ribosomal peptides, and hybrid and volatile metabolites.

Actinobacteria of the genus *Streptomyces* are dominant components of soil microbial communities and prolific producers of bioactive secondary metabolites. They account for a substantial proportion of clinically and agriculturally relevant antibiotics [30,31]. In agricultural systems, *Streptomyces* spp. suppress phytopathogenic fungi through competition, antibiosis, emission of volatile compounds, and secretion of cell wall–degrading enzymes [32–34]. The two primary classes of secondary metabolites produced by *Streptomyces* are non-ribosomal peptides and polyketides [35]. Several isolates have shown strong antagonistic activity against toxigenic *Fusarium* species and the potential to reduce mycotoxin accumulation [36].

In general, the literature indicates that *Trichoderma, Bacillus* and *Streptomyces* species possess complementary antifungal mechanisms, including enzymatic degradation of fungal cell walls, membrane damage and competition for nutrients. These properties underline their potential as key components of sustainable disease management strategies aimed at reducing reliance on chemical fungicides while maintaining crop productivity and health. The aim of this study was to evaluate the antifungal activity of selected *Streptomyces*, *Bacillus*, and *Trichoderma asperellum* strains against phytopathogenic fungi and to assess their potential as BCAs using in vitro assays.

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2. Materials and Methods

2.1. Microbial Strains

The antifungal activity of 14 selected microbial strains was evaluated against 16 strains of phytopathogenic fungi (Table 1). All strains were obtained from the Microbial Strain Collection of Latvia (MSCL) and maintained under standardized laboratory conditions.

Table 1. Microorganisms used in the study.

Species	MSCL number	Origin	Type of identification
Antagonists			
Bacillus subtilis	1441	Lupine soil, Latvia	biochemical
Bacillus subtilis	49	Unknown	biochemical
Bacillus stercoris	897	Commercial cleaner, Latvia	rRNA gene
Streptomyces anthocyanicus	420	Garden soil, Latvia	ANI
Streptomyces griseus	346	Garden soil, Latvia	rRNA gene, ANI
Streptomyces griseus	351	Garden soil, Latvia	rRNA gene, ANI
Streptomyces griseus	422	Garden soil, Latvia	ANI
Streptomyces griseus	424	Garden soil, Latvia	ANI
Streptomyces silvae	354	Garden soil, Latvia	rRNA gene
Streptomyces sp.	355	Garden soil, Latvia	rRNA gene
Streptomyces sp.	415	Garden soil, Latvia	micromorphological
Streptomyces sp.	349	Garden soil, Latvia	rRNA gene
Streptomyces venezuelae	350	Garden soil, Latvia	ANI
Trichoderma asperellum	309	Commercial preparation, Latvia	ITS
Pathogens			
Botrytis cinerea	433	Liver paste, Sweden	unknown
Colletotrichum salicis	850	Rhododendron leaves, Riga	ITS, LSU, TUB2, ACT, CHS-1, GAPDH, HIS3
Fusarium oxysporum	259	Unknown	unknown
Fusarium graminearum	435	Oat, Sweden	unknown
Fusarium culmorum	1690	*Avena sativa*, Latvia	micromorphological
Fusarium culmorum	1692	*Avena sativa*, Latvia	ITS, TEF
Fusarium culmorum	1693	*Avena sativa*, Latvia	ITS, TEF
Fusarium graminearum	1691	*Avena sativa*, Latvia	ITS, TEF
Fusarium graminearum	1694	*Avena sativa*, Latvia	ITS, TEF
Fusarium oxysporum	1696	*Avena sativa*, Latvia	micromorphological
Fusarium oxysporum	1699	*Avena sativa*, Latvia	ITS, TEF
Fusarium oxysporum	1700	*Avena sativa*, Latvia	ITS, TEF
Fusarium poae	1701	*Avena sativa*, Latvia	ITS, TEF
Fusarium sporotrichioides	1695	*Avena sativa*, Latvia	ITS, TEF
Fusarium sporotrichioides	1697	*Avena sativa*, Latvia	ITS, TEF
Fusarium tricinctum	1698	*Avena sativa*, Latvia	micromorphological

2.2. Culture Media and Growth Conditions

Fungi were cultured on Malt Extract Agar (MEA, Millipore, India), while bacterial strains were cultured on R2A Agar (Millipore, Germany). *B. subtilis* and *B. stercoris* were subcultured 3–4 days prior to testing to ensure active growth. Fungal cultures and *Streptomyces* were incubated for 7 days at 20–22 °C before antifungal assays.

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2.3. Dual Culture Assay

The antifungal activity of antagonist strains was evaluated using a dual culture assay. Agar discs (0.7 cm diameter) from actively growing colonies of phytopathogenic fungi and antagonist strains were placed 4 cm apart on MEA plates. Plates were incubated at 20–22 °C for 7 days. Antifungal activity was quantified by measuring fungal colony diameter and calculating the growth inhibition percentage: Percentage of inhibition = (colony diameter in control—colony diameter in treatment) x 100 / colony diameter in control [37]. Each fungus–antagonist combination was performed in triplicate.

2.4. Agar Well Diffusion Assay

The antifungal activity of *Bacillus* spp. strains was determined using an agar well diffusion assay. *Fusarium* suspensions were adjusted to an optical density of 0.16 at 540 nm and uniformly spread on MEA plates. Wells (0.7 cm diameter) were created in the agar and filled with 70 µL of *Bacillus* suspensions standardized to 0.16 at 540 nm. Plates were incubated at 20–22 °C for 7 days. Antifungal activity was expressed as inhibition zone diameter.

2.5. Assessment of Volatile and Soluble Metabolites of T. asperellum

To determine the contribution of volatile organic compounds (VOCs) produced by *T. asperellum*, a double-plate assay was performed according to [38]. *T. asperellum* and the pathogen were inoculated on the surface of agar plates in separate Petri dishes. The lids were removed and the bottom halves of the dishes were placed facing each other so that the inoculated agar surfaces were aligned. The paired dishes were sealed with several layers of Parafilm to prevent the escape of VOCs. Fungal growth inhibition was evaluated after 7 days of incubation at 20–22 °C.

The effect of soluble/diffusible metabolites of *T. asperellum* was evaluated using a cellophane overlay method [38]. *T. asperellum* was cultivated on MEA plates covered with sterile cellophane (Bright Ideas Crafts, UK) for 7 days. After removal of the fungal biomass and cellophane, *Fusarium* discs were transferred onto the conditioned medium. Colony diameter was measured after 48 h to 72 h at 20–22 °C, and growth inhibition percentage was calculated relative to untreated controls.

2.6. Statistical Analysis

All antifungal assays were performed in three independent replicates. Growth inhibition percentage and inhibition zone diameter were calculated as quantitative indicators of antifungal activity. Statistical analyses were conducted using RStudio (version 2024.12.1+563). One-way ANOVA was applied to evaluate the effect of antagonist strain on fungal growth inhibition. In experiments involving multiple phytopathogenic fungi, two-way ANOVA was used to assess the interaction between antagonist strain and fungal species. Duncan's multiple range test was applied for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Antifungal Activity of Trichoderma asperellum Against Fusarium spp.

3.1.1. Dual Culture Test

The antifungal activity of *T. asperellum* MSCL 309 against twelve phytopathogenic *Fusarium* isolates obtained from oats in Latvia was assessed using the dual culture assay. After seven days of incubation, growth inhibition was observed for all tested isolates. The growth inhibition ranged from 44.6% to 78.4% (Table 2, Figure 1), indicating substantial variability in isolate susceptibility. The highest inhibition, 71.0%—78.4%, was observed for both strains of *F. sporotrichioides* (MSCL 1697 and MSCL 1695), two strains of *F. oxysporum* (MSCL 1696 and MSCL 1700), *F. culmorum* MSCL 1693 and *F. poae* MSCL 1701. Both strains of *F. graminearum* (MSCL 1691 and MSCL 1694) exhibited significantly

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($p < 0.05$) lower sensitivity. The consistent inhibition across all isolates indicates that *T. asperellum* possesses broad-spectrum antifungal potential against *Fusarium* spp., although the degree of inhibition is species and/or isolate-dependent.

Table 2. Inhibitory effect of *T. asperellum* against twelve *Fusarium* strains. Numbers in a column followed by different letters are significantly different ($p < 0.05$).

***Fusarium* strains**	**Percentage of inhibition under dual culture conditions**
Fusarium culmorum MSCL 1690	49.1 ± 4.4^{c}
Fusarium graminearum MSCL 1691	44.6 ± 10.5^{c}
Fusarium culmorum MSCL 1692	46.7 ± 5.8^{c}
Fusarium culmorum MSCL 1693	71.4 ± 4.5^{a}
Fusarium graminearum MSCL 1694	48.9 ± 9.4^{b,c}
Fusarium sporotrichioides MSCL 1695	71.0 ± 3.9^{a}
Fusarium oxysporum MSCL 1696	74.4 ± 5.6^{a}
Fusarium sporotrichioides MSCL 1697	78.4 ± 7.0^{a}
Fusarium tricinctum MSCL 1698	56.7 ± 3.3^{b}
Fusarium oxysporum MSCL 1699	56.7 ± 6.6^{b}
Fusarium oxysporum MSCL 1700	74.1 ± 3.5^{a}
Fusarium poae MSCL 1701	71.0 ± 4.1^{a}

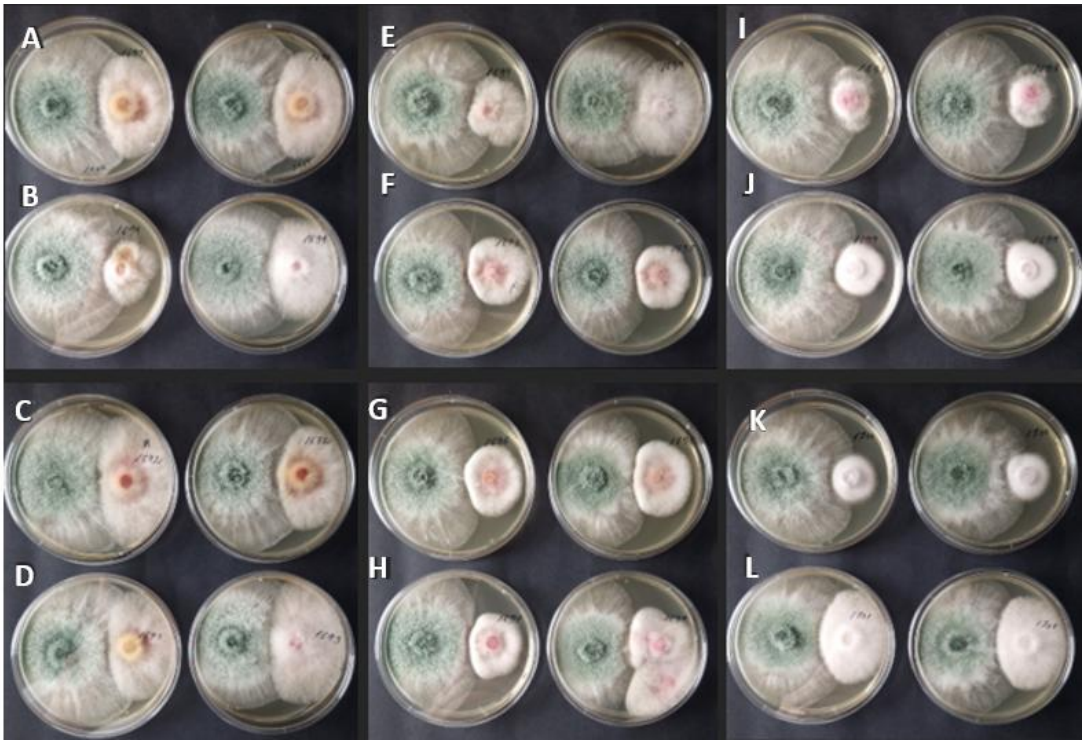

Figure 1. Dual culture test of *T. asperellum* MSCL 309 (on the left side of the Petri dishes) against (**A**) *Fusarium culmorum* MSCL 1690, (**B**) *F. graminearum* MSCL 1691, (**C**) *F. culmorum* MSCL 1692, (**D**) *F. culmorum* MSCL 1693, (**E**) *F. graminearum* MSCL 1694, (**F**) *F. sporotrichioides* MSCL 1695, (**G**) *F. oxysporum* MSCL 1696, (**H**) *F. sporotrichioides* MSCL 1697, (**I**) *F. tricinctum* MSCL 1698, (**J**) *F. oxysporum* MSCL 1699, (**K**) *F. oxysporum* MSCL 1700 and (**L**) *F. poae* MSCL 1701 (on the right side) after seven days of cultivation at 22-24 °C; two repetitions shown.

3.1.2. Contribution of Volatile Organic Compounds

The role of volatile organic compounds (VOCs) in providing antifungal activity was assessed separately using sealed plate assays. After 72 hours of incubation, no statistically significant differences ($p > 0.05$) were found between treated and control plates in terms of colony diameter,

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although slight variations in radial growth were visually observed for some isolates. These findings suggest that VOCs-mediated inhibition was negligible under the experimental conditions used. VOCs were either not synthesized in sufficient concentrations or did not have potent antifungal activity against the tested isolates in this experimental setting. It is also possible that environmental parameters, such as incubation time, headspace volume, or gas exchange conditions, limited VOC accumulation.

3.1.3. Contribution of Soluble Metabolites

In contrast to VOCs, soluble metabolites showed strong antifungal activity. After 48 hours of incubation, colony diameters on control plates ranged from 1.4 to 3.8 cm, while plates pretreated with soluble *T. asperellum* metabolites showed complete growth inhibition (Figure 2A). After 72 hours, the diameter of the control *Fusarium* colonies had increased 1.5–2-fold (Figure 2B). However, complete inhibition was still observed for isolates *F. graminearum* MSCL 1694, both strains of *F. sporotrichioides* (MSCL 1695 and MSCL 1697), *F. oxysporum* MSCL 1696, *F. tricinctum* MSCL 1698 and *F. poae* MSCL 1701 on pretreated plates. The other isolates also had significantly reduced colony diameters (1.0–1.6 cm), consistent with significant growth inhibition. These results demonstrate that soluble metabolites are the primary contributors to the antifungal activity of *T. asperellum* at least under in vitro conditions (Table 2, Figure 1).

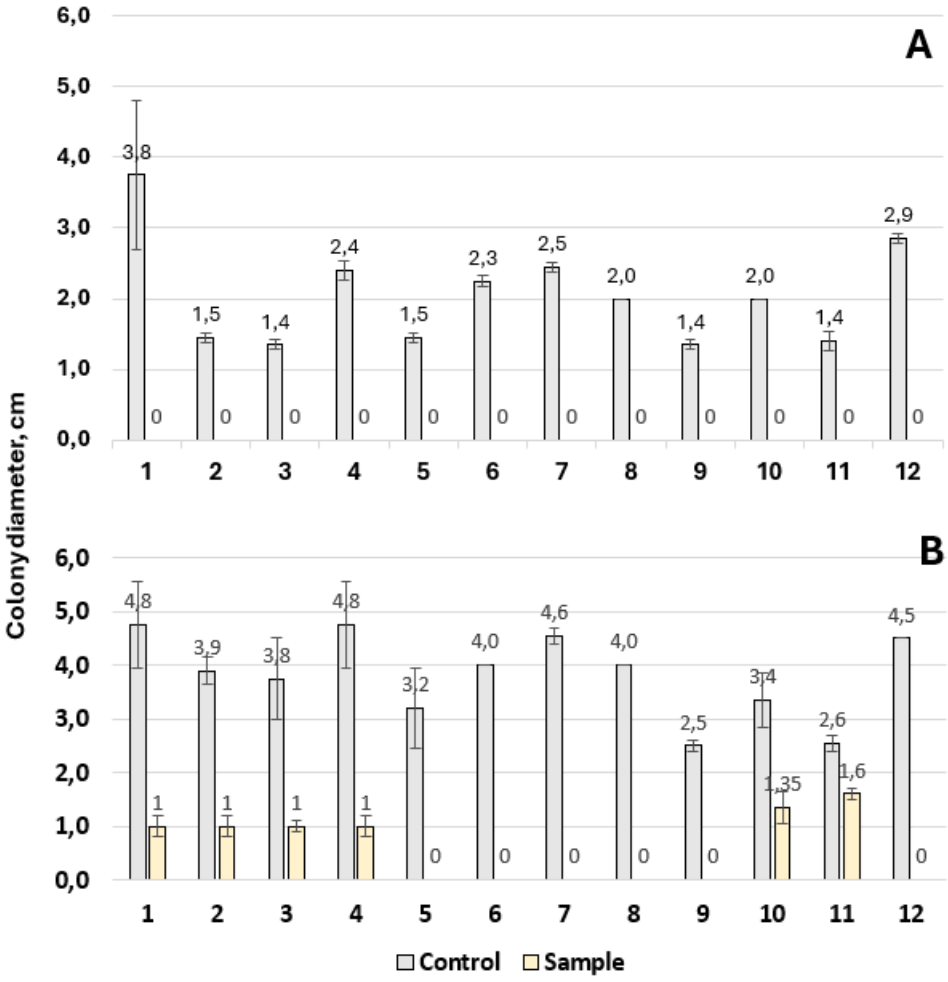

Figure 2. *Fusarium* colony diameter (cm ±SD) in plates without (Control) and with (Sample) *T. asperellum* solutes after 48 (**A**) and 72 hours (**B**) of cultivation. Strain transcription: 1. *Fusarium culmorum* MSCL 1690, 2. *F. graminearum* MSCL 1691, 3. *F. culmorum* MSCL 1692, 4. *F. culmorum* MSCL 1693, 5. *F. graminearum* MSCL 1694, 6. *F. sporotrichioides* MSCL 1695, 7. *F. oxysporum* MSCL 1696, 8. *F. sporotrichioides* MSCL 1697, 9. *F. tricinctum* MSCL 1698, 10. *F. oxysporum* MSCL 1699, 11. *F. oxysporum* MSCL 1700, and 12. *F. poae* MSCL 1701. Three replicates.

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3.2. Antifungal Activity of Bacillus species Against Fusarium spp.

An experiment conducted using two *B. subtilis* strains, MSCL 49 and MSCL 1441, and *B. stercoris* MSCL 897 to evaluate the antifungal activity of bacterial suspensions against twelve *Fusarium* spp. isolates showed antifungal activity for only one strain, *B. subtilis* 1441 (Figures 3 and 4). The largest diameter of the zone of inhibition was observed for both *F. graminearum* strains, MSCL 1694 and MSCL 1691, at 25 mm and 19 mm, respectively. The lowest inhibition was observed for *F. culmorum* MSCL 1690 (11 mm) and *F. sporotrichioides* MSCL 1697 (12 mm).

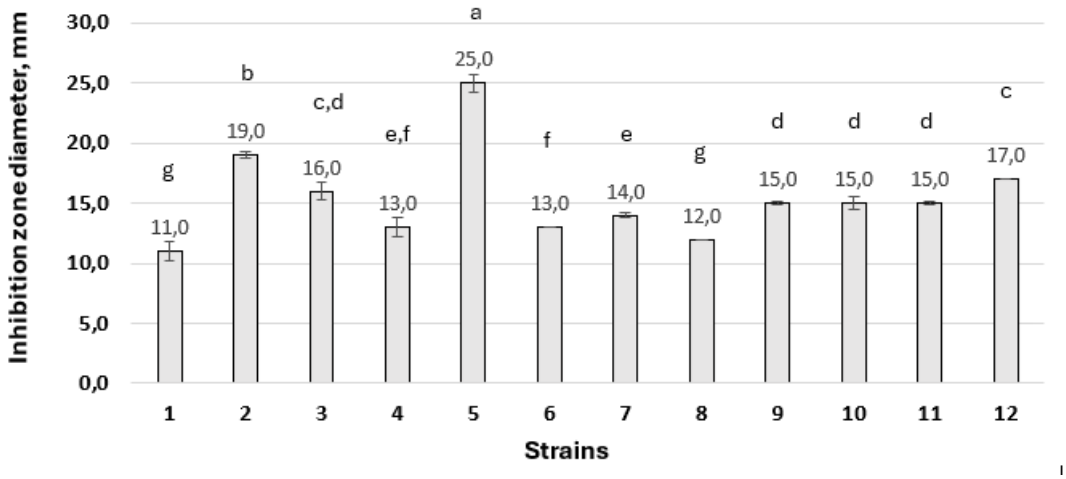

Figure 3. Diameter of the zone of inhibition of *Fusarium* (mm ± SD) after seven days of cultivation in agar well assay with *B. subtilis* MSCL 1441. Strain transcription: 1. *Fusarium culmorum* MSCL 1690, 2. *F. graminearum* MSCL 1691, 3. *F. culmorum* MSCL 1692, 4. *F. culmorum* MSCL 1693, 5. *F. graminearum* MSCL 1694, 6. *F. sporotrichioides* MSCL 1695, 7. *F. oxysporum* MSCL 1696, 8. *F. sporotrichioides* MSCL 1697, 9. *F. tricinctum* MSCL 1698, 10. *F. oxysporum* MSCL 1699, 11. *F. oxysporum* MSCL 1700, and 12. *F. poae* MSCL 1701. Numbers with different letters above the columns are significantly different ($p < 0.05$).

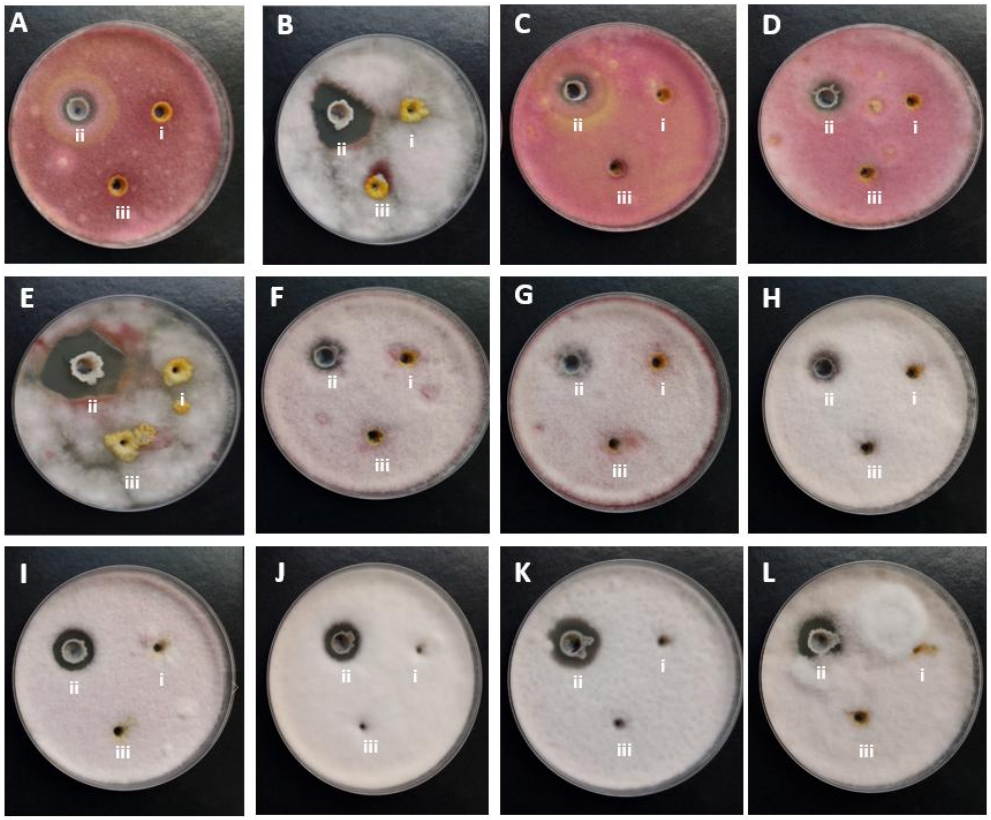

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Figure 4. Effect of three *Bacillus* strains plated in agar wells on the growth of *Fusarium* spp. after seven days of cultivation. i—*B. stercoris* MSCL 897, ii—*B. subtilis* MSCL 1441, iii—*B. subtilis* MSCL 49. (**A**) *Fusarium culmorum* MSCL 1690, (**B**) *F. graminearum* MSCL 1691, (**C**) *F. culmorum* MSCL 1692, (**D**) *F. culmorum* MSCL 1693, (**E**) *F. graminearum* MSCL 1694, (**F**) *F. sporotrichioides* MSCL 1695, (**G**) *F. oxysporum* MSCL 1696, (**H**) *F. sporotrichioides* MSCL 1697, (**I**) *F. tricinctum* MSCL 1698, (**J**) *F. oxysporum* MSCL 1699, (**K**) *F. oxysporum* MSCL 1700, and (**L**) *F. poae* MSCL 1701. One of three replicates is shown.

3.3. Antifungal Activity of Streptomyces spp. Against Phytopathogenic Fungi

Ten *Streptomyces* strains were evaluated against four phytopathogenic fungi using the dual culture assay. Antifungal activity varied markedly depending on both antagonist strain and fungal species (Figure 5). Across all tested *Streptomyces* strains, *S. venezuelae* MSCL 350 exhibited the highest antifungal activity against all pathogens, highlighting its superior antagonistic capacity. For *F. oxysporum*, the maximum inhibition zone diameter was 13.7 mm when paired with *S. venezuelae*. Other *Streptomyces* strains showed considerably weaker inhibition, ranging from 0.3 mm to 4.3 mm. *F. graminearum* demonstrated relatively high resistance, and significant inhibition was observed only in *S. venezuelae* (18.0 mm), while inhibition was minimal or absent for most other strains.

Botrytis cinerea showed moderate sensitivity. The strongest inhibition was again observed in *S. venezuelae* (20.7 mm), while the other strains produced inhibition zone diameters ranging from 1.3 mm to 4.3 mm.

The most sensitive species tested was *Colletotrichum salicis*. The diameter of the inhibition zone for *S. venezuelae* reached 22.7 mm, and other *Streptomyces* strains also caused significant inhibition (7.0–9.7 mm). This suggests a greater sensitivity of *C. salicis* to actinobacterial antagonism compared to *F. graminearum* and *B. cinerea*.

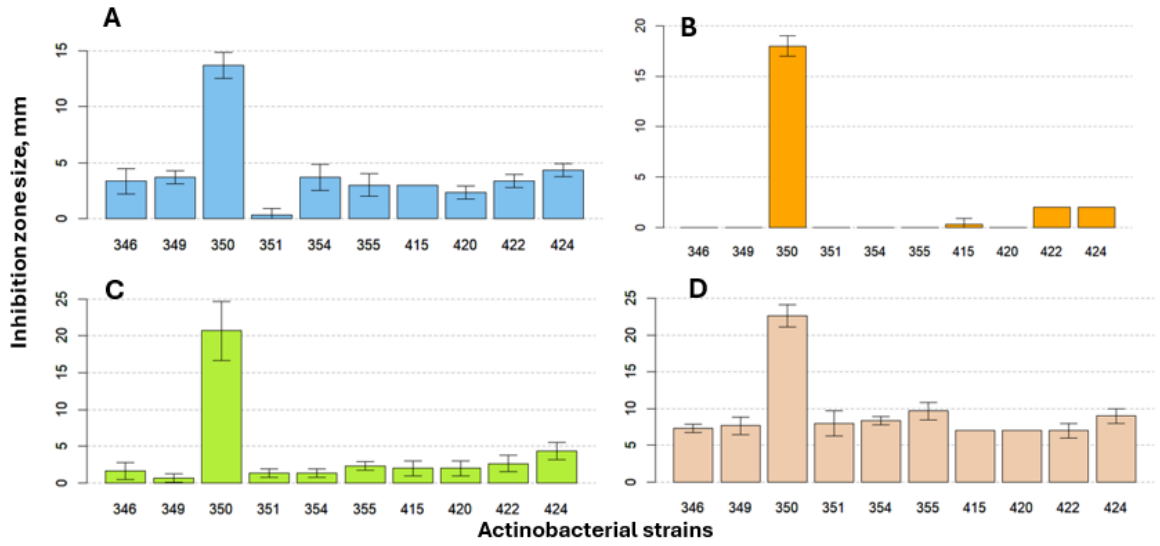

Figure 5. Inhibition zone size (mm ±SD) of *Fusarium oxysporum* (**A**), *F. graminearum* (**B**), *Botrytis cinerea* (**C**) and *Colletotrichum salicis* (**D**) depending on the opposite growing actinobacteria *Streptomyces griseus* MSCL 346, *Streptomyces* sp. MSCL 349, *S. venezuelae* MSCL 350, *S. griseus* MSCL 351, *S. sylvae* MSCL 354, *Streptomyces sp.* MSCL 355, *Streptomyces* sp. MSCL 415, *S. anthocyanicus* MSCL 420, *S. griseus* MSCL 422 and *S. griseus* MSCL 424, in three replicates after seven days of incubation.

Two-way ANOVA analysis confirmed statistically significant differences in fungal sensitivity ($p < 0.05$), supporting the conclusion that antifungal activity depends not only on antagonist strain but also on intrinsic properties of the phytopathogenic fungus. These findings emphasize the importance of strain selection in BCAs applications and suggest that *S. venezuelae* MSCL 350 represents a particularly promising candidate for further development.

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4. Discussion

The present study demonstrates that antifungal activity is strongly dependent on both the antagonist strain and the phytopathogen species and strain. Among the tested microorganisms, *Trichoderma asperellum* MSCL 309 and *Streptomyces venezuelae* MSCL 350 exhibited the highest and most consistent antifungal activity, whereas investigated *Bacillus* spp. showed comparatively moderate effects.

4.1. Antifungal Mechanisms of Trichoderma asperellum

The dual culture assay revealed broad-spectrum inhibition of all tested *Fusarium* isolates by *T. asperellum*, supporting previous reports describing *Trichoderma* spp. as effective antagonists of soil-borne phytopathogens [17,18]. Experiments show that of the *Fusarium* species studied, *F. graminearum* is relatively difficult to control (Table 1). The observed isolate-dependent growth inhibition percentage is consistent with documented variability in susceptibility among *Fusarium* species and strains [1,2].

The absence of statistically significant inhibition in VOCs assay suggests that VOCs played a limited role under the applied in vitro conditions. Although *Trichoderma* spp., including *T. asperellum*, are known to produce VOCs with antifungal properties [39], their efficacy may depend on environmental conditions and metabolite concentration [20]. The present findings suggest that the antagonism mediated by VOCs was negligible compared to other metabolites.

In contrast, soluble metabolites demonstrated strong antifungal activity, including complete inhibition of several isolates. This observation aligns with established mechanisms of *Trichoderma* antagonism, which involve secretion of hydrolytic enzymes (chitinases, β-1,3-glucanases) and secondary metabolites that degrade fungal cell walls or disrupt membrane integrity [19,20]. Such enzymatic and metabolite-driven inhibition likely represents the primary mechanism of antifungal activity observed in this study.

4.2. Strain-Specific Antifungal Activity of Bacillus spp.

B. subtilis MSCL 1441 showed significant antifungal activity, compared to the *B. subtilis* MSCL 49 and *B. stercoris* MSCL 897, confirming the strain-dependent nature of the antagonism. *Bacillus* spp. are widely recognized for producing cyclic lipopeptides such as iturins, surfactins, and fengycins, which disrupt fungal cell membranes [24,25]. It has been found that both the studied *B. subtilis* MSCL 1441 and *B. stercoris* MSCL 897 synthesize surfactins and fengycins, but not iturins [40], however, the antagonistic activity differed significantly, indicating the involvement of other metabolites. *B. stercoris* belongs to the "*subtilis* group" and "*subtilis* subgroup" [41]. It was originally identified as *B. subtilis* subsp. s*tercoris*, but in 2020, based on genomic analysis, it was elevated to the species level [42].

Additionally, *Bacillus* spp. may contribute to pathogen inhibition through siderophore-mediated iron competition and induction of plant defense responses [26,27]. However, under the strictly in vitro conditions of this study, the dominant mechanism was likely direct metabolite-mediated inhibition.

B. subtilis MSCL 1441 stood out among all other potential antagonists in that it was able to strongly inhibit the *F. graminearum* species (Figure 3), which was relatively resistant to the others.

4.3. Superior Antagonistic Potential of Streptomyces venezuelae

The dual culture assay demonstrated that antifungal activity among *Streptomyces* spp. was species or strain-dependent, with *S. venezuelae* MSCL 350 producing the largest inhibition zone diameters. This is consistent with the well-documented capacity of *Streptomyces* spp. to synthesize diverse bioactive secondary metabolites, including antibiotics with antifungal properties [30,31]. The strong inhibition observed in dual culture assay suggests that *S. venezuelae* MSCL 350 secretes extracellular antifungal metabolites. Similar findings have been reported for other *Streptomyces*

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strains capable of suppressing *Fusarium* spp. and other phytopathogenic fungi via antibiotic production [33,34].

Soil bacterium *S. venezuelae* is known as an antibiotic (chloramphenicol, jadomycin and pikromycins) producer and differs from many other *Streptomyces* in its ability to complete its entire life cycle in liquid culture [43,44]. It is possible that this is what allowed this bacterium to display properties of its extracellular metabolites that were not possible for other *Streptomyces* under liquid cultivation. The expression of secondary metabolite biosynthesis gene clusters in *Streptomyces* spp. is associated with the complex morphological differentiation of the bacteria during the transition from vegetative to aerial mycelium and subsequent sporulation, therefore these genes are called silent or cryptic, and under laboratory conditions they are usually weakly expressed [45].

Species-dependent sensitivity was evident in this study. *Colletotrichum salicis* showed the highest sensitivity (Figure 5D), while *Fusarium graminearum* showed relatively higher resistance (Figure 5B), as in the case of *T. asperellum* (Table 1). Such differences may be linked to intrinsic pathogenicity mechanisms, cell wall architecture, and adaptive stress responses [6,7].

The consistent activity of *S. venezuelae* MSCL 350 against multiple phytopathogenic fungi suggests its potential as a broad-spectrum BCA.

5. Conclusions

The present study demonstrates that antifungal activity against phytopathogenic fungi is strongly dependent on both the antagonist strain and the pathogen species. Of the microorganisms tested, *T. asperellum* MSCL 309 and *S. venezuelae* MSCL 350 showed the greatest antifungal activity, while *B. subtilis* MSCL 1441 was the most potent specifically against *F. graminearum* strains.

T. asperellum MSCL 309 displayed broad-spectrum inhibition against all tested *Fusarium* isolates. The results indicate that soluble metabolites, rather than VOCs, are the primary contributors to antifungal activity under in vitro conditions.

Within the tested actinobacteria, *S. venezuelae* MSCL 350 demonstrated superior antagonistic performance. The consistent inhibition observed across multiple phytopathogenic fungi suggests strong secretion of extracellular antifungal metabolites. In contrast, antifungal activity among *Bacillus* strains was strain-dependent, with only *Bacillus subtilis* MSCL 1441 showing significant inhibition.

Species and strain-dependent sensitivity was evident among phytopathogenic fungi, highlighting the necessity for targeted biocontrol strategies. The variability in growth inhibition percentage emphasizes the importance of selecting highly effective antagonist strains rather than relying solely on genus-level identification.

From a practical perspective, the results identify *T. asperellum* MSCL 309, *S. venezuelae* MSCL 350 and *B. subtilis* MSCL 1441 as promising candidates for the development of biological control formulations aimed at managing phytopathogenic fungi, particularly *Fusarium* spp. Future studies should focus on molecular and chemical characterization of antifungal metabolites and evaluation of the performance of the new BCAs under greenhouse and field conditions to assess real-world efficacy and plant growth–promoting potential.

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Abbreviations

The following abbreviations are used in this manuscript:

BCA	biocontrol agent
FHB	*Fusarium* head blight
MSCL	Microbial Strain Collection of Latvia
VOCs	volatile organic compounds

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