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Biosurfactant-producing *Bacillus* spp. suppress *Fusarium* via fungal membrane disruption and promote cucumber growth

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Abstract

The increasing demand for sustainable agricultural practices has led to the exploration of beneficial microorganisms that promote plant growth and enhance resistance to phytopathogens. In this study, we characterized the Kol B9 strain isolated from the rhizosphere of *Vinca minor*. Morphological, biochemical, and molecular analyses were used to identify the strain as a member of the genus *Bacillus*, belonging to the *Bacillus subtilis* group. The strain exhibited strong antagonistic activity against *Fusarium culmorum* DSM 1094 and *Fusarium sambucinum IM 6525,* both on solid and in liquid media. This activity coincided with the production of surface-active cyclic lipopeptides and was accompanied by alterations in fungal membrane lipid composition, increased membrane permeability, and inhibition of spore germination and mycelial development. The environmental isolate *F. sambucinum* IM 6525 was less sensitive to *Bacillus* activity. In addition, *B. subtilis* Kol B9 promoted cucumber seedling growth and reduced the adverse effects of *Fusarium* infection. These findings support the potential application of *B. subtilis* Kol B9 as a bioinoculant in sustainable agriculture.

Keywords

Bacillus subtilis; surfactin, iturin, cyclic lipopeptides; plant growth promotion; *Fusarium* spp.; biocontrol; rhizosphere

Introduction

Bacillus spp. are widely present across diverse environments such as soil, water, plant surfaces,

and animal digestive tracts, where they participate in organic matter turnover and nutrient cycling [1]. The ability of *Bacillus* to form endospore, tolerate stress, and exhibit metabolic versatility enables their persistence in heterogenous habitats and frequent association with plants [2]. In plant-associated niches, *Bacillus* spp. establish mutualistic interactions that can be exploited for biological control and plant growth promotion.

The plant growth–promoting effects of *Bacillus* spp. are associated with multiple mechanisms, including phytohormone production, modulation of root system architecture, and improved nutrient acquisition. They also serve as biocontrol agents by suppressing soil-borne pathogens through various mechanisms. These mechanisms are primarily categorized into direct antagonism such as parasitism, antibiosis, and competition and indirect biocontrol activities, which involve the induction of systemic resistance (ISR) mechanisms in plants [3]. The mechanisms of biological control of soil-borne pathogens by *Bacillus* spp. are discussed by Sulaiman and Bello [4] and Fira et al. [5]. Due to all of these abilities, *Bacillus* bacteria are classified as plant growth-promoting microorganisms (PGPM) [6].

Among the metabolites contributing to both growth promotion and antifungal activity, cyclic lipopeptides (cLPs) produced by *Bacillus* spp. play a central role. Due to their amphiphilic and surface-active properties, cLPs are classified as biosurfactants and are synthesized by nonribosomal peptide synthetases, resulting in considerable structural diversity. According to Pecci et al. [7], *Bacillus* cLPs (cLPs-Bc) can be classified as homologues (differing in the length of the fatty acid chain) or isoforms (characterized by a different sequence of amino acids in the peptide part). Environmental *Bacillus* strains usually secrete biosurfactants in the form of a mixture of different cLPs homologues and/or isomers. The diversity and percentage composition of different cLPs variants produced by *Bacillus* strains depends, among others, on the composition of the microbiological media used, the culture conditions and pollutants presence [8-11]. The cLP-Bc comprise three major families: surfactins, iturins, and fengycins. These compounds can interact with fungal membrane lipid components, which may result in increased membrane permeability and leakage of cellular contents, as supported by recent studies linking cLP activity with fungal membrane remodeling and lipid composition changes [12, 13]. Additionally, cLPs-Bc differ from other antimicrobial compounds by their multifunctionality, acting not only as membrane-active agents but also as key mediators of rhizosphere interactions, biofilm formation, root colonization, and plant defense signaling [14-18]. In addition to lipopeptides, *Bacillus* spp. produce a broad spectrum of secondary metabolites, including polyketides (e.g., difficidin, macrolactin), volatile organic compounds, and enzymes (e.g., chitinases, glucanases). These compounds can act synergistically to suppress

fungal pathogens and indirectly improve nutrient acquisition by plants through enhanced root development and rhizosphere interactions, as well as promote plant defense responses. Recent studies further highlight the importance of metabolite synergy and environmental context in shaping *Bacillus*-mediated biocontrol efficacy, reinforcing the potential of *Bacillus* spp. as effective plant growth-promoting and biocontrol agents in sustainable agriculture [19-22].

Although numerous studies have demonstrated the biocontrol potential of *Bacillus* spp. against *Fusarium* pathogens, most of them rely on model strains or isolates maintained in culture collections. Consequently, the relevance of these interactions under natural rhizosphere conditions remains insufficiently explored. In particular, limited attention has been paid to the responses of environmental *Fusarium* isolates that may exhibit increased tolerance due to long-term exposure to microbial competition in soil.

Moreover, while cyclic lipopeptides produced by *Bacillus* spp. are widely recognized as key contributors to antifungal activity, their association with fungal membrane responses and developmental processes has rarely been examined in an integrated manner and linked directly to plant performance. Addressing these gaps is essential for improving the ecological relevance of microbial biocontrol studies and for developing effective, environmentally friendly alternatives to chemical fungicides.

Therefore, the objectives of this study were to evaluate the antagonistic potential of biosurfactant-producing *Bacillus* strains against *Fusarium* spp.; to investigate fungal cellular responses associated with membrane integrity, lipid composition, and developmental processes in response to treatment with the selected strain *Bacillus subtilis* Kol B9; and to assess the effects of *B. subtilis* Kol B9 on cucumber seedling growth and protection against *Fusarium* infection, with particular emphasis on interactions involving an environmental rhizospheric isolate of *F. sambucinum.*

Materials and Methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and are specified in the relevant methodological sections. Unless stated otherwise, media components, solvents, and standards were obtained from Sigma-Aldrich (St. Louis, MO, USA), POCH (Gliwice, Poland), or Chempur (Piekary Śląskie, Poland).

Microbial strains

All of the bacterial strains used in the study were isolated from non-urban areas, precisely from rhizosphere of *Vinca minor* (Kol B2, Kol B9), *Pinus sylvestris* (Kol S8), *Picea abies* (Kol Si4, Kol Si8) and from the vicinity of a rural roadside environment (Kol D3, Kol D8) and stored in the strain collection of the Department of Industrial Microbiology and Biotechnology, University of Lodz, Poland. In all cases, only rhizospheric soil was collected, without disturbing or removing any plant material. The plant species were identified in the field based on characteristic morphological features and habitat. None of the plant species present at the sampling locations are legally protected in Poland (according to the Regulation of the Polish Minister of the Environment of October 9, 2014, on the protection of plant species), and the sampling sites were located in the home garden, non-protected areas.

The *Fusarium* strains used in this study included *F. culmorum* DSM 1094, originally isolated from the rhizospheric soil of infected *Triticum aestivum* L. and obtained from the Leibniz Institute DSMZ (Germany), and *Fusarium sambucinum* IM 6525, isolated from the rhizospheric soil of infected *Rubus idaeus* L. and obtained from the strain collection of the National Institute of Horticultural Research (Skierniewice, Poland).

Bacteria identification

Molecular identification of *Bacillus* sp. B9 was carried out by the Department of Microbiology, Microbiological Quality Testing Laboratory and Laboratory of Biotechnology and Molecular Engineering (Warsaw, Poland). Genomic DNA was extracted using the DNeasy® PowerFood® Microbial Kit (Qiagen), and the 16S rDNA region was amplified with universal primers (16S-F, 16S-R). DNA sequencing was performed by Genomed S.A. and analyzed using BLAST against GenBank database entries.

Phenotypic identification was conducted using the Biolog system (Biolog Inc.). Gram-positive, rod-shaped bacteria were identified using GENIII test (Biolog Inc., cat. no. 1030). Briefly, the strain was cultured on R2A medium for 48 hours at 30 °C. The GENIII test plate was then inoculated with the bacteria biomass suspended in inoculation fluid B (Biolog Inc, cat. no. 72402), in accordance with the manufacturer's instructions. The inoculated plate was incubated at 30 °C for 48 hours, with readings taken every 24 hours. The resulting biochemical patterns were interpreted using Micrologsoftware (version 5.2.01) and GENIII database (version 2.7.1).

Antifungal activity assessment

Growth inhibition analysis

Antifungal activity of *Bacillus* strains was assessed using dual cultures grown on PDA medium as well as liquid cultures grown in LB medium (Sigma-Aldrich, St. Louis, MO, USA). For dual cultures mycelial discs with 8 mm diameter were cut from the fungi colonies cultured on PDA medium for seven days and placed onto fresh PDA plates. Then, bacteria collected from a 24-h-old culture conducted on a agar solidified broth medium were spot-inoculated on the opposite side of the plate. The control setup involved mycelium cultivated without bacteria. Samples were then incubated in the dark, at 28 °C, until the growing control mycelium covered the entire surface of the PDA medium. The SketchAndCalc™ software (iCalc, Inc., USA) was used to determine the area occupied by the mycelium, and then the percentage of inhibition of the growth of the mycelium incubated in the presence of bacteria was calculated in relation to the growth of the control mycelium (assumed to be 100%). Antifungal activity was also assessed in liquid LB medium. Briefly, 20 mL of LB medium was inoculated with 1% of 24-hour-old *Bacillus* culture and 10% of 48-hour-old *Fusarium* culture. Cultures were incubated at 28 °C with 140-rpm rotary shaking for 72 hours, followed by vacuum filtration through 0.22 µm filter papers (Millipore Sigma, Billerica, MA, USA). The mycelium pellet was dried at 60°C until a constant mass was obtained. Fungal growth in liquid cultures was expressed as corrected dry mycelial biomass, calculated by subtracting the dry bacterial biomass measured in parallel monocultures from the total biomass obtained in the co-cultures. Antifungal activity was assessed based on mycelium biomass and expressed as a percentage of the control culture biomass (cultivated without bacteria), taken as 100%. Assays were carried out in triplicates.

Spore germination and hyphae condition analysis

In order to obtain a suspension of *Fusarium* spores, the surface of the fungal culture (grown on agar-solidified broth medium) was washed with sterile distilled water containing 0.005% (v/v) Tween 20 (Sigma-Aldrich, St. Louis, MO, USA). The obtained suspension was filtered through sterile glass wool to remove mycelial hyphae. Spores were counted using a Thoma chamber, and the spore content in 1 mL of the sample was determined. In order to determine the effect of *Bacillus* metabolites on spore germination, the supernatant obtained after centrifugation (10,000 rpm, 15 min) of the 72-hour-old bacterial culture was further filtered through a membrane syringe filter with pore diameter 0.2 µm (Bionovo, Legnica, Poland). An aqueous suspension of spores (with a previously determined cell density) was added to the supernatant prepared in this way using a 1:1 (v/v) ratio. The sample was incubated for 48 hours in static conditions at 22-24 °C. Microscopic analysis of the morphology of spores and hyphae was performed using a Nikon Eclipse 50i light microscope equipped with a Nikon DS-Fi3 camera

and NIS-Elements D 5.11.01 software (Nikon Corporation, Tokyo, Japan). The obtained results were expressed as the intensity of spore germination and related to the number of spores present in the control sample, which was prepared by mixing an aqueous suspension of spores with LB medium (1:1, v/v). SketchAndCalc™ software was used to measure the length of the hyphae.

Fungal membranes permeability

To assess the changes in the membrane permeability of fungal cells growing in the presence of bacteria, the method described by Jasińska et al. [23] was used. For this purpose, 1 mL of liquid culture was centrifuged (12,000 rpm, 10 min). Then, the post-culture liquid was discarded, and the mycelium was resuspended in 1 mL of phosphate-buffered saline buffer (PBS; Sigma-Aldrich, St. Louis, MO, USA) at pH 7.4. To this, 2 µL of an aqueous solution of a 0.1 mg mL^{-1} propidium iodide (Sigma-Aldrich, St. Louis, MO, USA) was added to the pellet. Next, the samples were incubated for 5 min at room temperature in the dark, then centrifuged, and the supernatant was discarded. The mycelium was washed twice with PBS, resuspended in 1 mL of PBS, and transferred to a 24-well plate.

Fluorescence measurements were carried out using a FLUOstar Omega spectrofluorometer (BMG Labtech, Ortenberg, Germany) with the following parameters: λ_{ex} = 540 − 10 nm, λ_{em} = 630 − 10 nm, gain = 2000. The obtained results were related to the fluorescence of the supernatant obtained after centrifugation of the test sample (12,000 rpm, 10 min). The mycelial biomass present in the sample was then dried to a constant mass and weighed. The results were shown as a fluorescence unit (U) per 1 mg of the fungal biomass, taking the result obtained for the control system as 100%.

Lipidomic study

To 100 mg of wet biomass obtained from the fungal cultures conducted in LB medium (with or without bacteria), 1 mm glass beads and 1 mL of a 3:1 (v/v) mixture of tert-butyl methyl ether (POCH, Gliwice, Poland) and methanol (POCH, Gliwice, Poland) were added. The sample was then placed in a MM400 ball mill (Retsch, Haan, Germany) and the cells were disintegrated by shaking at 30 vibrations/s for 8 min. Next, 0.2 mL of ultrapure H_2O was added, and the samples were centrifuged at 7,200 rpm for 3 min. Then the upper layer was transferred, evaporated, and the obtained precipitate was dissolved in 1 mL of methanol (Sigma-Aldrich, St. Louis, MO, USA) and analyzed by LC–MS/MS technique using ExionLC AC UHPLC (Sciex, USA) coupled with Sciex QTRAP 4500 tandem mass spectrometer. A Kinetex C18 column (50 mm × 2.1 mm, particle size: 5 µm; Phenomenex, Torrance, CA, USA) was used under the following

conditions: 40 °C temperature and a mobile phase flow rate 500 µL min^{-1}. The ion source of the mass spectrometer was operated in negative mode. Phospholipid analyses were performed using Analyst™ v1.6.3 software (Sciex, USA) [24].

Biosurfactant content analysis

The surface activity of post-culture fluids of *Bacillus* spp. was assessed using a modified drop collapse test (DCT) [25]. For this purpose, 10 µL of post-culture liquid obtained after centrifugation of liquid culture (10,000 rpm, 10 min) was placed on a polypropylene surface and the droplet diameter was measured after 1 hour. As negative and positive controls, 10 µL of LB medium and 5% aqueous sodium dodecyl sulfate (SDS; Chempur, Piekary Śląskie, Poland) solution were used, respectively.

cLP-Bc isolation from liquid cultures was performed using a modified extraction method of QuEChERS [9]. The extraction was performed using the post-culture liquid obtained after centrifugation of the culture (10,000 rpm, 15 min). The process included six stages: 1) 5 mL of culture supernatant and 5 mL of distilled water were placed in a 50 mL Falcon tube; 2) 10 mL of acetronitrile (ACN; Chempur, Piekary Śląskie, Poland) was added to the mixture and shaken vigorously for 2 min; 3) a four salt mixture was added (2 g $MgSO_4$ (Sigma-Aldrich, St. Louis, MO, USA), 0.5 g NaCl (POCH, Gliwice, Poland), 0.5 g $C_6H_5NaO_7 \times 2H_2O$ (Biomus Łódź, Poland), 0.25 g $C_6H_6Na_2O_7 \times 1.5\ H_2O$ (Biomus, Łódź, Poland)) and the mixture was shaken for 5 min; 4) the sample was left to stand for 20 min; 5) the organic phase (upper layer) was collected for analysis; 6) another 10 mL portion of ACN was added to the sample and the extraction procedure was repeated.

cLP-Bc was analyzed using LC–MS/MS (LC 1200 coupled to a Sciex QTRAP 3200 tandem mass spectrometer, Agilent Technologies, Santa Clara, CA, USA) equipped with an ESI source. Compounds were separated on a Kinetex C18 column (50 mm × 2.1 mm, particle size: 5 µm) (Phenomenex, Torrance, CA, USA) maintained at 40 °C. The mobile phase used for compound separation consisted of water (A) and methanol (B) (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 5 mM ammonium formate (Sigma-Aldrich, St. Louis, MO, USA). The flow rate used was 600 µL min^{-1}. The analysis time was 6 min. The solvent gradient was started from 40% B, increased to 100% B after 0.5 min in 1.5 min, which was then maintained for 2.5 min. The initial solvent composition was returned within 2 min. The ion source of the mass spectrometer was operated in positive mode. Data were analyzed using the software Analyst™ v1.5.3 (Sciex). The surfactin and iturin standards (Sigma-Aldrich, St. Louis, MO, USA) were used in the studies. For sodium $(M + Na)^+$ surfactin homologues C13, C14, C15 and C16, the

monitored MRM pairs were m/z 1030–391, 1044–391, 1058–391 and 1072–391. For sodium ions of iturin A homologues C13, C14, C15 and C16, the monitored MRM pairs were m/z 1051.5/1051, 1065.5/1065, 1079.5/1079 and 1093.5/1093.

The presence of fengycin was confirmed using MALDI–TOF/TOF 5800 (Sciex, Framingham, MA, USA). A mixture of 0.5 µL of cLP-Bc extract (diluted in 2 mL of methanol) and 0.5 µL of a solution containing 10 mg mL^{-1} 2,5-dihydroxybenzoic acid (DHB; Sigma-Aldrich, St. Louis, MO, USA) dissolved in ACN was applied to the MALDI plate. MALDI–TOF/TOF analyses were performed in positive ionization mode in the 33 m/z range of 900–2000 at a constant laser intensity of 3500. The ten most intense signals were selected for automatic MS/MS measurement at a constant laser intensity of 5000.

Plant growth promotion

Initial Plant Growth in Phytotoxkit Plates

To evaluate the initial growth of roots and hypocotyls, cucumber seeds were surface-sterilized with sodium hypochlorite and subsequently inoculated with either distilled water or a standardized bacterial suspension adjusted to 1×10^8 CFU mL^{-1}. The suspension was thoroughly mixed prior to inoculation to ensure uniform cell concentration. The plates were lined with filter paper moistened with bacterial suspension, whereas the filter paper was moistened with deionized water in the control plates. The seeds were placed in Phytotoxkit plates (Phytotoxkit, Wageningen, Netherlands), arranged in a single line with even spacing, and ten seeds were placed per plate at approximately 1 cm from the central edge. The plate covers were closed starting from the center to prevent seed displacement. The Phytotoxkit plates containing the seeds were maintained at 20 °C in accordance with the Phytotoxkit protocol, which reflects conditions suitable for seedling development. The experiment was conducted in four replicates. The Phytotoxkit plates were positioned vertically in a tray, and images of the plates in a horizontal position were captured daily using a digital camera. Root and hypocotyl lengths were measured using dedicated image analysis software. At the end of the experiment, roots and hypocotyls were scanned separately using an EPSON EXPRESSION 10000 XL scanner (Epson, Suwa, Japan). Root and hypocotyl growth characteristics were determined using WinRhizo software (Regent Instruments, Quebec, Canada) to measure root length, root surface area, root diameter, root volume, and the number of root tips.

Pot experiment

Soil (containing high peat, compost, calcium carbonate, guano, horn flour; pH value 5.0-7.0; Compo Bio, Poland) was placed in pots. Distilled water or an aqueous suspension of *Fusarium* spores (prepared according to the method described in section "Spore germination and hyphae condition analysis") was added to the soil in such a volume that it constituted 1% of the soil volume. Cucumber seeds (*Cucumis sativus* L.) were surface-sterilized with 5% NaClO and incubated for 3 hours in a bacterial suspension (24-h LB culture) or in water under shaking (90 rpm, 22 °C). After removing the suspension, seeds were placed on sterile filter paper in Petri dishes and incubated in the dark for 24 hours at 22–24 °C. Then, cucumber seeds were sown at a depth of approx. 2 cm. Each test system contained 60 cucumber seeds (six replicates of 10 seeds each). The samples were incubated for 120 hours at 22-24 °C, under diffused sunlight. Then, the roots were separated from the stems and dried to a constant weight. The average root mass per seedling was calculated and the results were compared with the control group (assumed to be 100%), in which the seeds were incubated in the soil in the absence of microorganisms.

Statistical Analysis

All experiments were conducted at least in triplicate. The obtained results were presented as their mean values. An average standard deviation (SD) was calculated ($n \geq 3$). One-way ANOVA analysis of variance was performed using Microsoft® Excel® for Microsoft 365 MSO, version 2401 (Microsoft Corporation, Washington, DC, USA). Differences at $p \leq 0.05$ were considered significant.

3. Results

3.1. Screening of lipopetides-producing *Bacillus* spp. with antifungal activity

Bacillus spp. strains isolated from non-urban soil were investigated for their biosurfactant activity, as well as their antifungal and plant growth-promoting potential. In the first phase of the study, the surface activity of biosurfactants was assessed after 72 hours of cultivation in LB medium using the DCT assay (Table 1). To further characterize biosurfactant composition, cLPs were isolated using the QuEChERS method, followed by quantitative analysis using LC–MS/MS and MALDI–TOF/TOF techniques. The results showed that all of the tested strains exhibited high surface activity, with DCT values ranging from 8 to 9.5 mm, indicating their significant capability to produce biosurfactants. Nearly all of the tested strains produced surfactin, iturin and fengycin. Only in *Bacillus* sp. Kol Si6 cultures fengycin was not detected. The highest iturin production (exceeding 70 mg L^{-1}) was found in the cultures of strains Kol B2, Kol D3 and Kol D8. In contrast, the highest levels of surfactin (above 200 mg L^{-1}) were produced by strains Kol B2, Kol B9, Kol D8, Kol Si4, and KolSi6.

Table 1. Biosurfactant production by rhizosphere *Bacillus* strains after 72 h of growth in LB medium under shaking conditions at 28 °C.

Strain of bacteria	DCT [mm]*	cLP		
		Surfactin [mg L^{-1}]	Iturin [mg L^{-1}]	Fengycin [+/-]
Kol B2	8	94.8 ± 16.5	289.7 ± 43.8	+
Kol B9	9	39.5 ± 6.0	205.1 ± 31.0	+
Kol D3	9	71.3 ± 10.8	172.5 ± 26.0	+
Kol D8	9,5	92.4 ± 13.9	236.0 ± 35.7	+
Kol S8	9,5	32.2 ± 4.1	169.3 ± 25.6	+
Kol Si4	8	36.8 ± 5.5	420.1 ± 63.5	+
Kol Si6	8	22.7 ± 3.4	379.3 ± 57.3	-

* DCT assay results for LB medium (negative control) and 5% SDS aqueous solution (positive control) were 3 and 8 mm, respectively. The standard deviation for the DCT result of the tested systems was 1.5 mm.

Table 2 reports the mycelium growth intensity of *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525 (expressed as a percentage relative to the control culture cultivated without bacteria) in the presence of various bacterial strains cultivated on both solid and in liquid media. All tested bacterial strains inhibited the growth of the *Fusarium* fungi to some extent. The inhibitory effect was significantly more pronounced when microorganisms were grown in liquid medium, due to the direct physical interaction between the microorganisms. Among the two fungal strains, *F. culmorum* DSM 1094 exhibited greater sensitivity to the tested bacteria. Notably, strains Kol B9 and Kol Si6 limited its growth by over 80%. The above-mentioned strains also showed strong antagonistic activity towards the second of the tested fungal strain, *F. sambucinum* IM 6525, limiting its growth by 66 and 80.6%, respectively. Figure 1 displays macro- and microscopic photos illustrating this interaction, using Kol B9 strain in co-culture with *F. sambucinum* IM 6525 as an example. Macroscopic observations of the growth of *F. sambucinum* IM 6525 co-cultured with bacterial strains on solid medium revealed an intensive, dendritic growth of the bacterial colonies towards the parent bacterial colony (Fig. 1A). This directional growth effectively formed a physical barrier, preventing the fungal hyphae from further colonizing the medium. As a result, the fungus was also restricted from accessing essential nutrients and elements from the medium, further inhibiting its development and spread. Microscopic analysis of the peripheral mycelium showed bacterial adhesion to the surface of the hyphae (Fig. 1B). Colonization of mycelium by bacterial cells was observed for all of the tested *Bacillus* strains.

Table 2. Growth intensity of mycelium of *F. sambucinum* IM 6525 and *F. culmorum* DSM 1094 cultivated on solid medium and in liquid medium in the presence of *Bacillus* spp. or (dual culture) for 120 h.

Strain of bacteria	**Growth intensity of mycelium [%] cultivated for 120 h**			
	on solid medium		**in liquid medium**	
	***F. sambucinum* IM 6525**	***F. culmorum* DSM 1094**	***F. sambucinum* IM 6525**	***F. culmorum* DSM 1094**
Without bacteria (control)	100	100	100	100
Kol B2	60	44,1	50,7	59,2

Kol B9	60	68,5	34	19,2
Kol D3	60	62,1	23,6	26,7
Kol D8	60	58	75,9	66,1
Kol S8	62,5	61,9	28,2	24
Kol Si4	62,6	52	19,9	26,1
Kol Si6	70,9	54,3	19,4	18,2

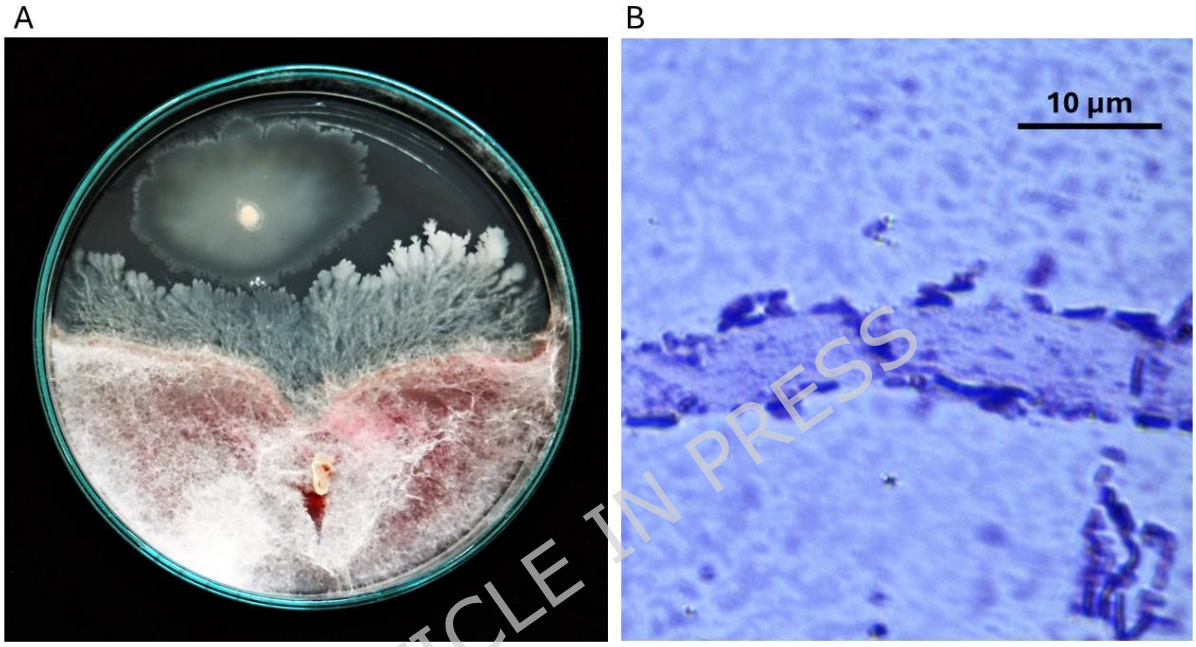


Figure 1. Dual culture of mycelium of *F. sambucinum* IM 6525 and *Bacillus* sp. Kol B9 bacteria grown on solid PDA medium for 120 h of incubation. A) macroscopic evaluation; and B) microscopic image of a crystal violet-stained preparation containing marginal mycelium.

The differences in fungal growth suppression among the strains suggest variations in their antifungal mechanisms, possibly related to biosurfactant or other secondary metabolites production. To investigate this further, the concentration of lipopeptides produced by bacteria was assessed (Fig. 2). In the majority of cultures, the presence of fungal mycelium led to a significant (over 50%) decrease in the levels of isolated biosurfactants. However, only in the cultures of *Bacillus* sp. Kol D3 and *Bacillus* sp. Kol B9, the presence of *F. culmorum* DSM 1094 resulted in an increase in iturin levels by 10 and 20%, respectively. Notably, surfactin levels did not increase in any of the *Bacillus-Fusarium* co-cultures. Only in the culture containing *Bacillus* sp. Kol D8 and *F. sambucinum* IM 6525 the level of surfactin was comparable to the level determined for the control (bacterial monoculture).

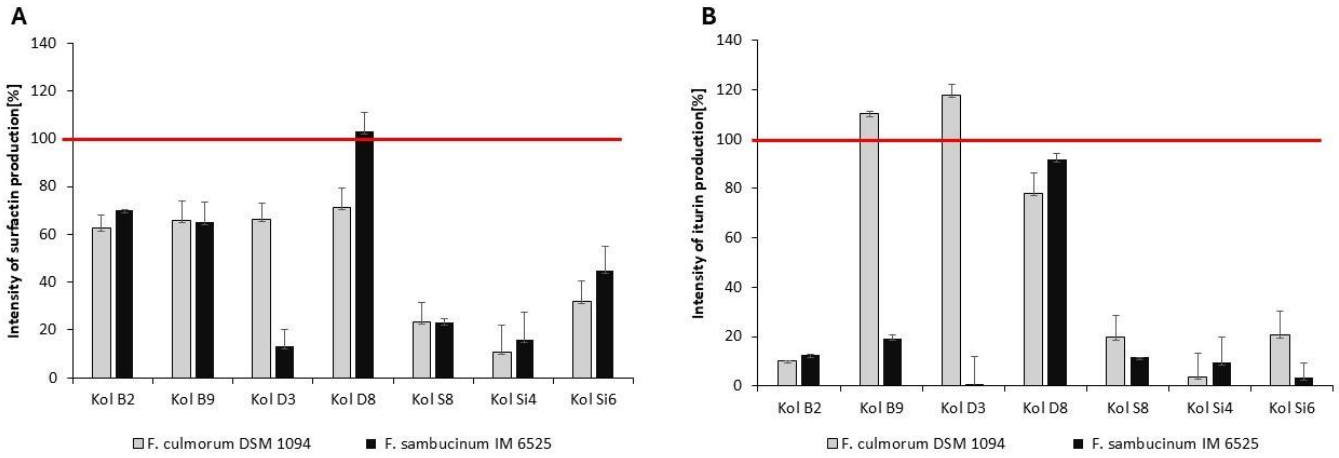


Figure 2. Intensity of A) surfactin and B) iturin production (%) determined in 72-hour-old mixed bacterial-fungal co-cultures containing mycelia of *F. culmorum* DSM 1094 or *F. sambucinum* IM 6525. The red line indicates the level of biosurfactant production in control cultures (carried out in the absence of mycelia) taken as 100%. Error bars represent standard deviation (SD).

3.2. Evaluation of the cellular response of *Fusarium* sp. strains to *B. subtilis* Kol B9

In the next stage of the study, the cellular response of the tested fungal strains to *Bacillus* sp. Kol B9 was investigated. The *Bacillus* sp. Kol B9 strain was identified using molecular techniques. Analysis of the 16S rDNA operon region revealed 100% sequence identity with several species: *B. amyloliquefaciens*, *B. siamensis*, *B. velezensis*an, and *B. subtilis*. Due to the high sequence similarity within the *Bacillus* genus, species-level identification based solely on 16S rDNA analysis is not conclusive.

However, based on the Kol B9 strain's biochemical profile -including its ability to oxidize various carbon sources and its metabolic activity (synthesis of dehydrogenases) in presence of antibiotics, varying NaCl concentrations, and different pH levels after 24 and 48 hours of incubation - the strain Kol B9 was excluded as a member of *B. velezensis* and assigned to the *Bacillus subtilis* group (Table S1, S2).

3.2.1. Spore germination and hyphal length

The inhibition of fungal mycelial growth and spore germination is a well-established mechanism by which biocontrol agents suppress phytopathogens. Therefore, the effect of *B. subtilis* Kol B9 culture on *Fusarium* mycelial growth and conidial germination was evaluated.

The obtained results showed that bacterial metabolites present in the culture supernatants had a significant effect on both spores germination and average mycelial length. In the presence of the bacterial supernatant, only 60% of *F. sambucinum* IM 6525 and 42% of *F. culmorum* DSM 1094 spores germinated, compared to the control (Figure 3A, Figure 4). The inhibitory effect of *B. subtilis* Kol B9 metabolites was also reflected in the length of *F. sambucinum* IM 6525 and *F. culmorum* DSM 1094 hyphae. In untreated control samples, the average hyphal lengths for *F. sambucinum* IM 6525 and *F. culmorum* DSM 1094 were 843 and 1444 µm, respectively. However, in the cultures exposed to bacterial metabolites, the hyphal length was significantly reduced to 102 µm (Figure 3B).

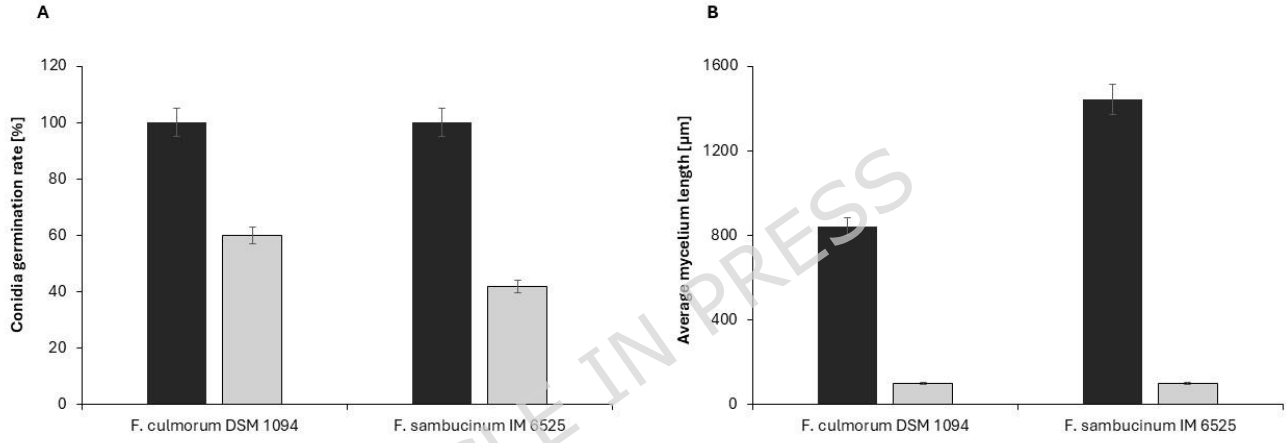


Figure 3. *F. sambucinum* IM 6525 and *F. culmorum* DSM 1094 conidia germination [%] and average mycelium length [µm] without bacteria (black) and in the presence of *B. subtilis* Kol B9 supernatant (grey). Error bars represent standard deviation (SD).

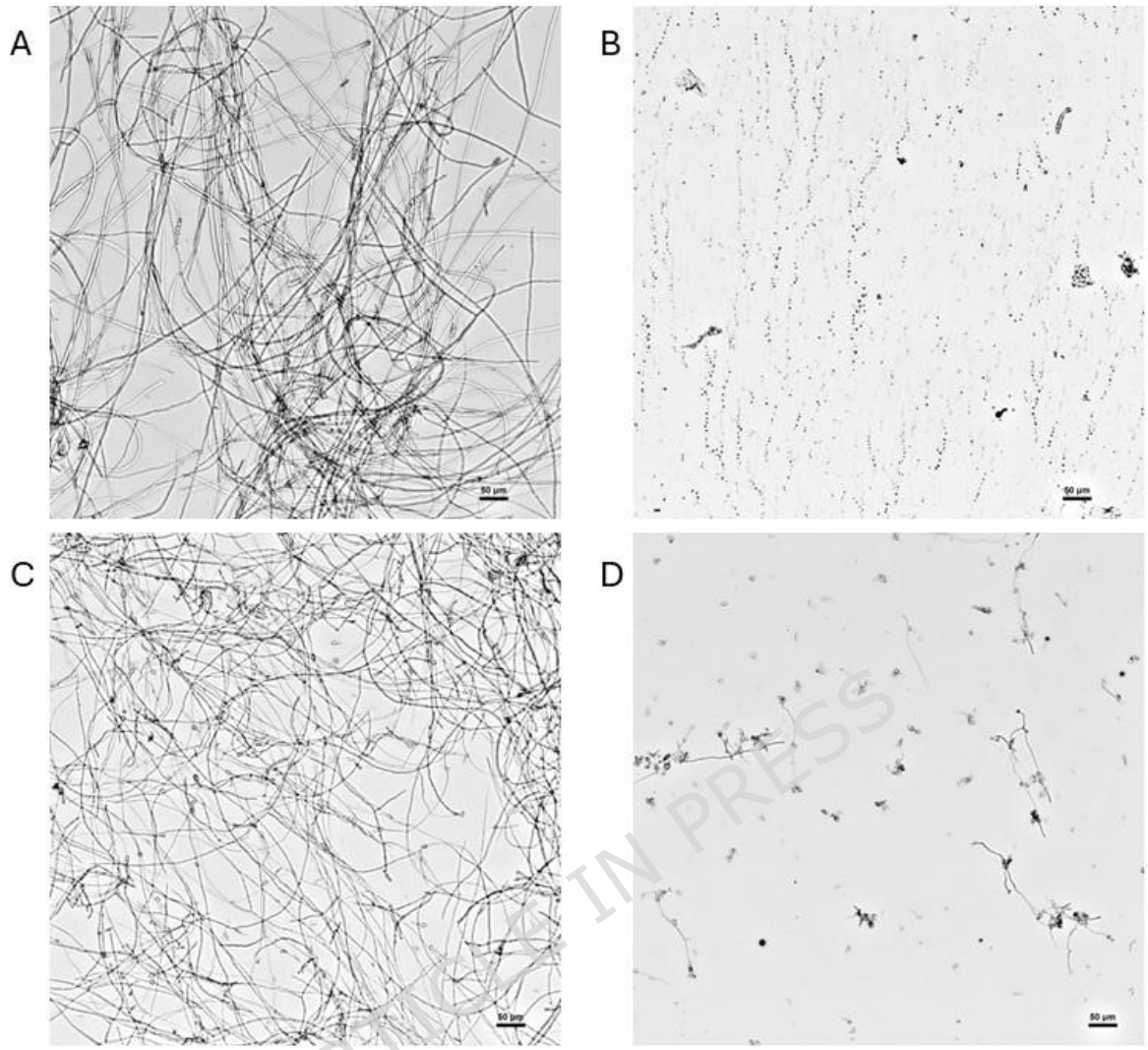


Figure 4. Germination of mycelium spores of *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525 in the control cultures (A and C, respectively) or in the presence of post-culture fluids of *B. subtilis* Kol B9 (B and D).

3.2.2. Fungal lipidome

Changes in the mycelial lipidome were assessed by qualitative and quantitative analysis of phospholipids using biomass collected from 72-hour-old liquid cultures, including both monocultures of *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525, as well as mixed bacterial-fungal cultures with *B. subtilis* Kol B9. The studied *Fusarium* strains responded differently to the presence of *B. subtilis* Kol B9 in the growth environment (Table 3). In the *F. culmorum* DSM 1094 biomass, a decrease in the content of the dominant phospholipid classes — phosphatidylcholine (PC) and phosphatidylethanolamine (PE) — was observed, dropping from 52.95% and 47.05% to 40.77% and 37.77%, respectively. Simultaneously, a significant

amount of phosphatidylglycerol (PG), comprising 21.47% of total phospholipids, was detected. Notably, PG was absent in the biomass of the control cultures. Despite these changes, the PC:PE ratio — an indicator of membrane integrity — remained largely unaffected. In the mycelium of *F. sambucinum* IM 6525, a decrease in the content of PC (from 60.66 to 50.68%) and a simultaneous increase in the PE content (from 39.31 to 45.51%) were noted, resulting in a markedly altered PC:PE ratio. This result aligns with observations from the spore germination assay, where more pronounced effects of *B. subtilis* Kol B9 were observed for *F. culmorum* DSM 1094.

Table 3. Percentage of phospholipids isolated from biomass samples obtained from 72-hour liquid cultures of *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525 grown without additives and in the presence of *B. subtilis* Kol B9 bacteria.

	Relative Abundance [%]			
	***F. culmorum* DSM 1094**	***F. culmorum* DSM 1094 with *B. subtilis* Kol B9**	***F. sambucinum* IM 6525**	***F. sambucinum* IM 6525 with *B. subtilis* Kol B9**
PC	52.95	40.77	60.66	50.68
PE	47.05	37.77	39.31	45.51
PG	0.01	21.47	0.04	3.8
PC/PE	1:0.9	1:0.8	1:0.6	1:0.9

Detailed analysis of individual phospholipid species revealed that the lipidomic changes in *F. culmorum* DSM 1094, induced by *B. subtilis* Kol B9, were particularly pronounced (Fig. 5). The most prevalent phospholipid species - PC 18:2/18:2, representing approximately 40% of the total phospholipid pool - decreased by half in the presence of the bacteria. Similar reductions were also observed for PC 16:0/18:2 and PE 18:3/18:2. The content of PE 18:2/18:2 in the presence of bacteria was three times lower than in the control system (without bacteria addition). In turn, a significant increase (3-19 fold) in content of phospholipids was observed for PC 16:0/18:1, PC 18:1/18:1, PE 16:0/18:1, and PE 18:1/18:1. In the mycelium of *F. sambucinum* IM 6525, a notable decrease in the main PC species - PC 18:2/18:2- was accompanied by simultaneous increase of the content of 8 out of 11 detected PE species.

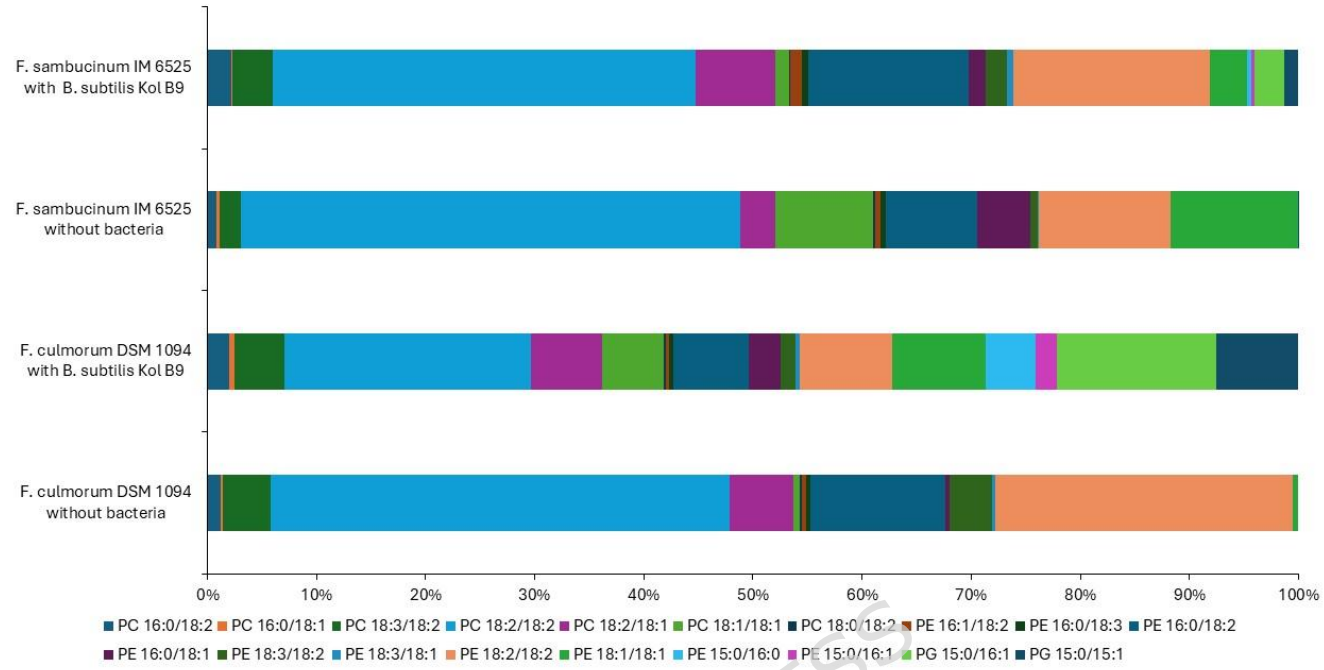


Figure 5. Comparison of phospholipid composition (percentage of the total phospholipids from species) of *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525 cultured without or with the presence of *B. subtilis* Kol B9.

3.2.3. Fungal cell membrane permeability

It was found that the mycelia of both of the tested *Fusarium* strains cultivated in the presence of the bacterial strain, exhibited increased cell membrane permeability compared to the control (Figure 6). The permeability of cell membrane, expressed as propidium iodide fluorescence, was significantly higher in *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525 cells cultivated with *B. subtilis* Kol B9, reaching 5066 and 2129 U·mg^{-1} of dry biomass, respectively. This was 5 and 6 times greater than the membrane permeability noted in fungal cells grown without the bacteria.

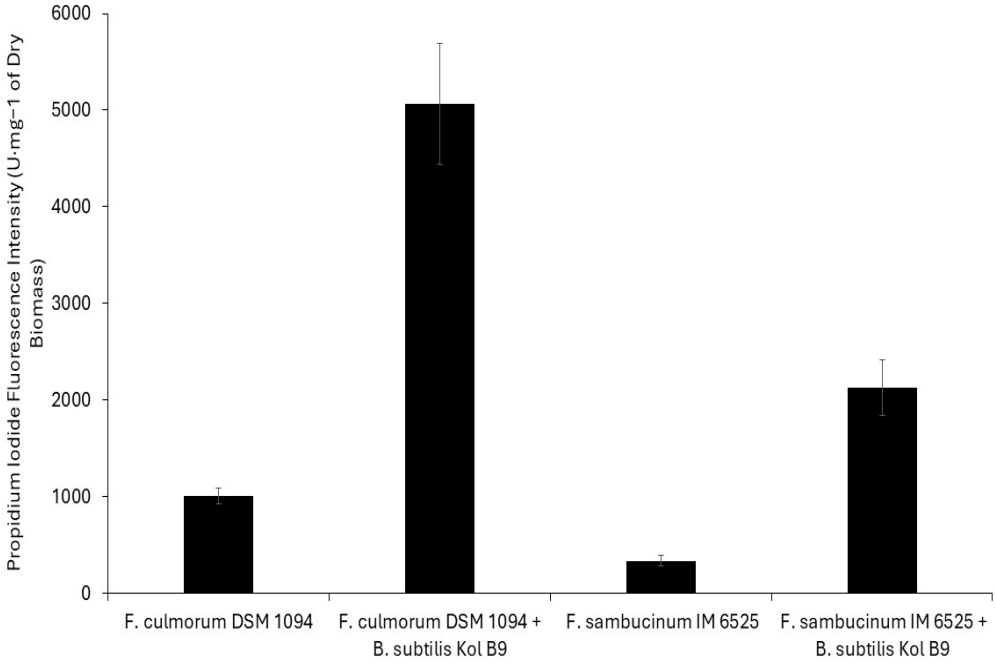


Figure 6. Membrane permeability (expressed as propidium iodide fluorescence in fungal biomass) of *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525 cells cultivated with or without *B. subtilis* Kol B9 for 72 hours. Error bars represent standard deviation (SD).

3.3. Analysis of root health and plant growth promotion

A detailed analysis of cucumber root health and growth promotion was performed using *Bacillus* sp. Kol B9 strain. The results indicated that seed germination commenced 24 h after imbibition (Fig. 7). Inoculation of cucumber seeds with *B. subtilis* Kol B9 bacteria positively influenced the dynamics of roots and hypocotyls growth. A visible positive response to seed inoculation with *B. subtilis* Kol B9 bacteria was observed three days after their imbibition. After six days, the roots obtained from seeds treated with *B. subtilis* Kol B9 were 25 mm longer than the control ones grown from seeds inoculated in H_2O (Fig. 7). Hypocotyls emerged after four days of incubation.

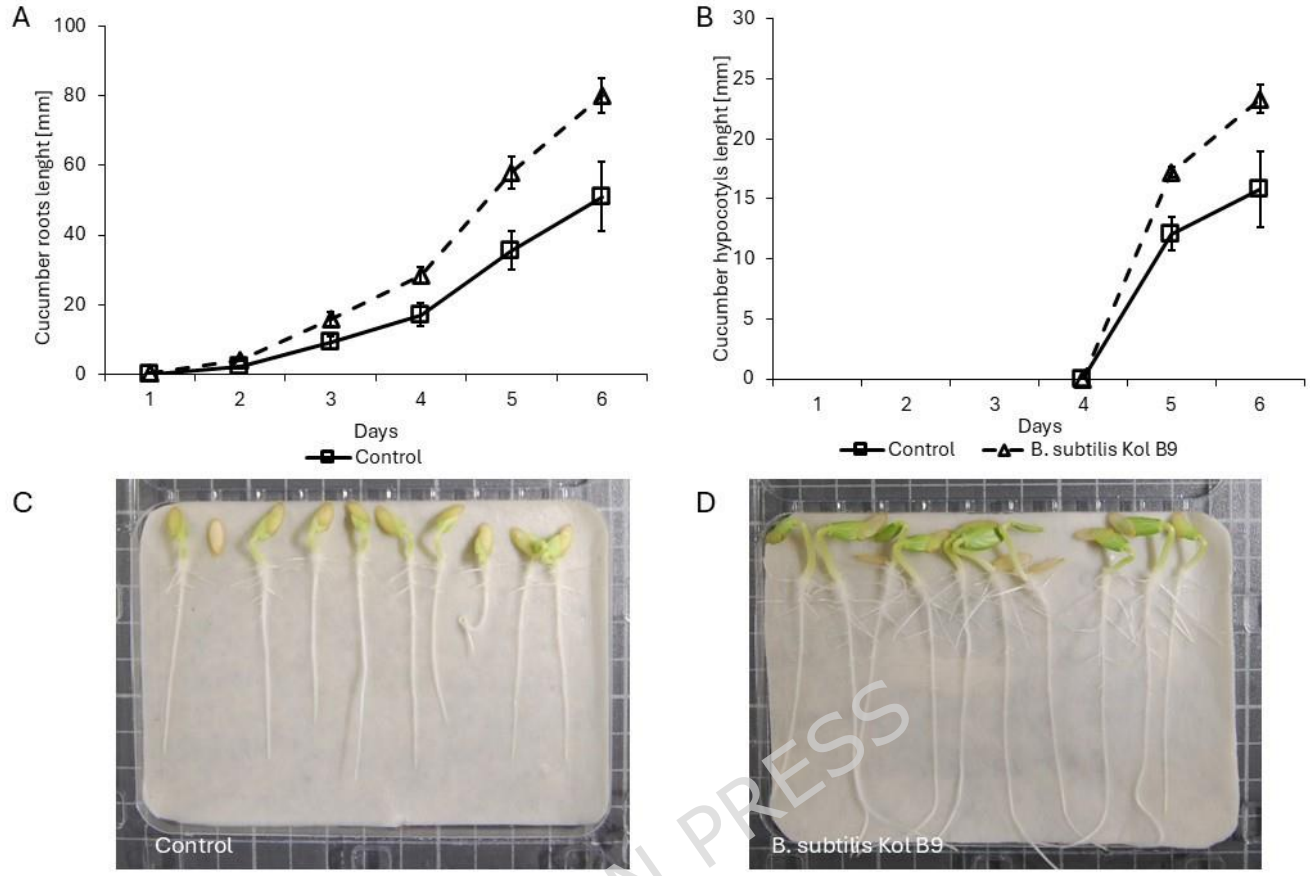


Figure 7. The dynamics of cucumber roots (A) and hypocotyls growth (B) obtained from seeds inoculated in distilled water (control) and bacteria *B. subtilis* Kol B9. Cucumber seedlings after six days of imbibition in Phytotoxkit plates. Control seedlings (C) were derived from seeds inoculated with water, while treated seedlings (D) originated from seeds inoculated with *B. subtilis* Kol B9. Error bars represent standard deviation (SD).

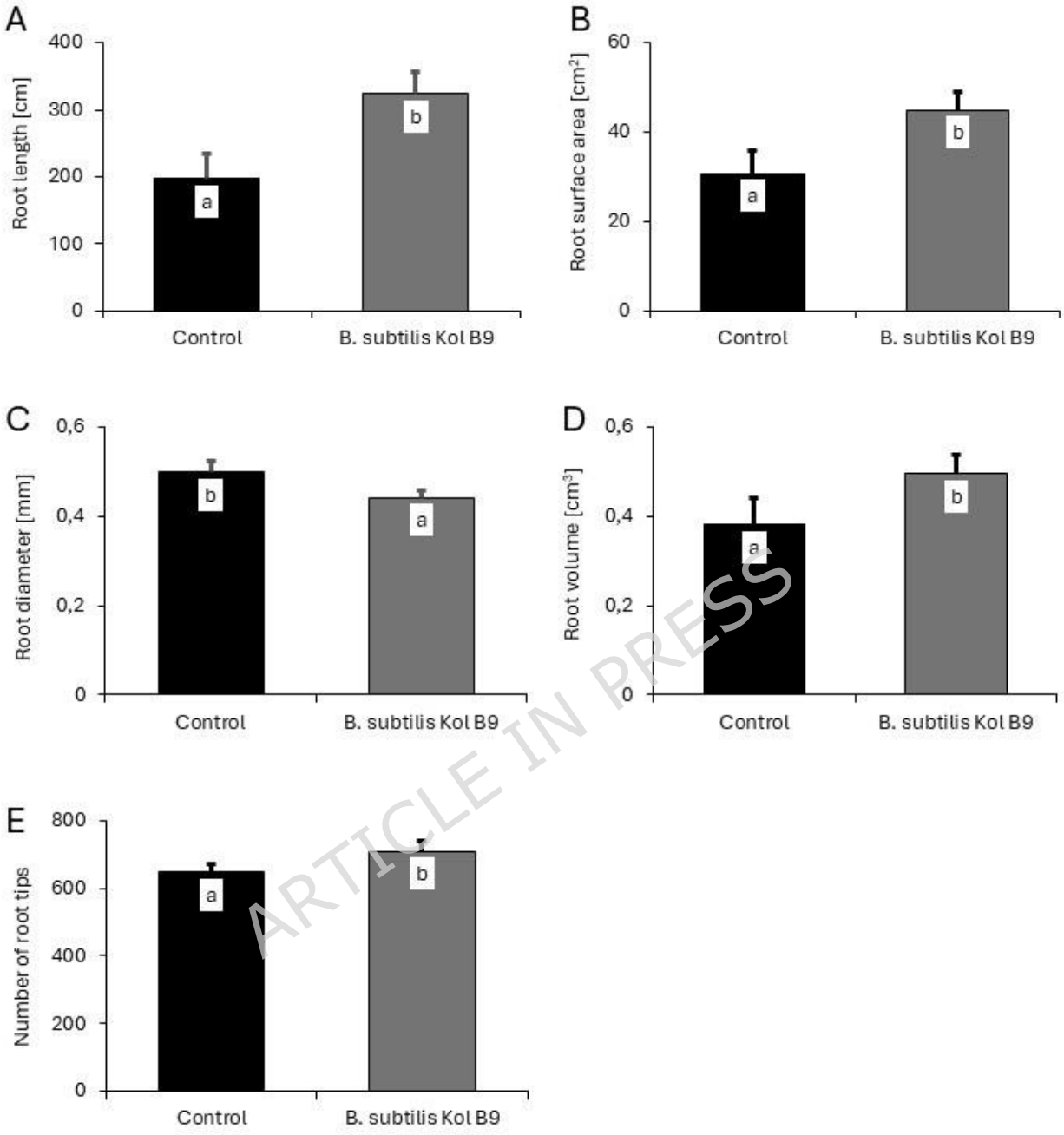


Figure 8. The root length (A), root surface area (B), root diameter (C), root volume (D), and the number of root tips (E) of cucumber seedlings grown from seeds inoculated in distilled water (control) or the strain of *B. subtilis* Kol B9. Error bars represent standard deviation (SD).

The presented data demonstrate that inoculation with the *B. subtilis* Kol B9 strain significantly increased the length, surface area, volume of cucumber roots, as well as the number of root tips

(Fig. 8). However, despite these positive effects, the roots of Kol B9-treated seedlings exhibited a smaller diameter (0.44 mm) compared to the control (0.50 mm).

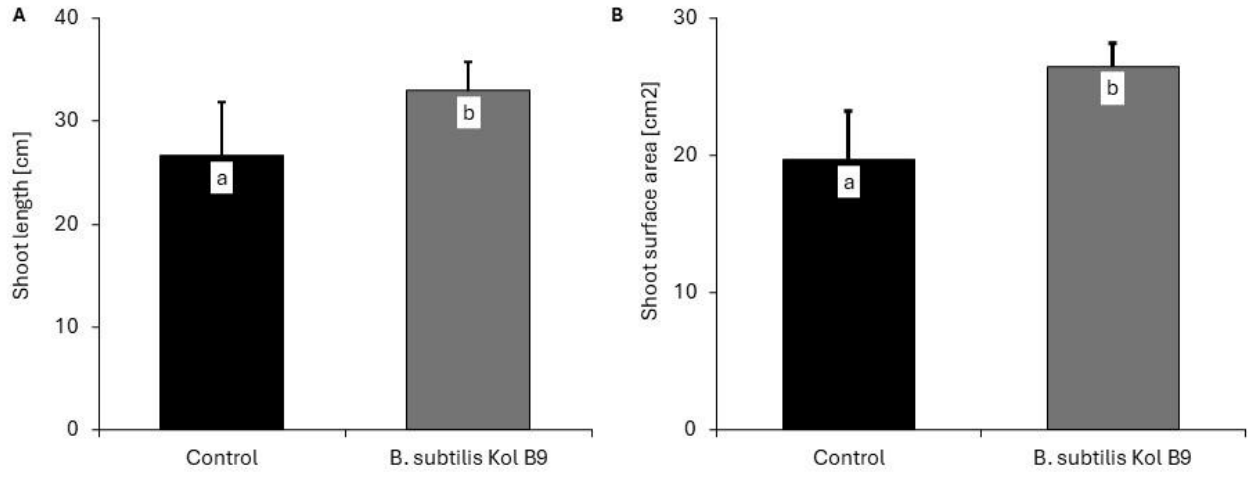
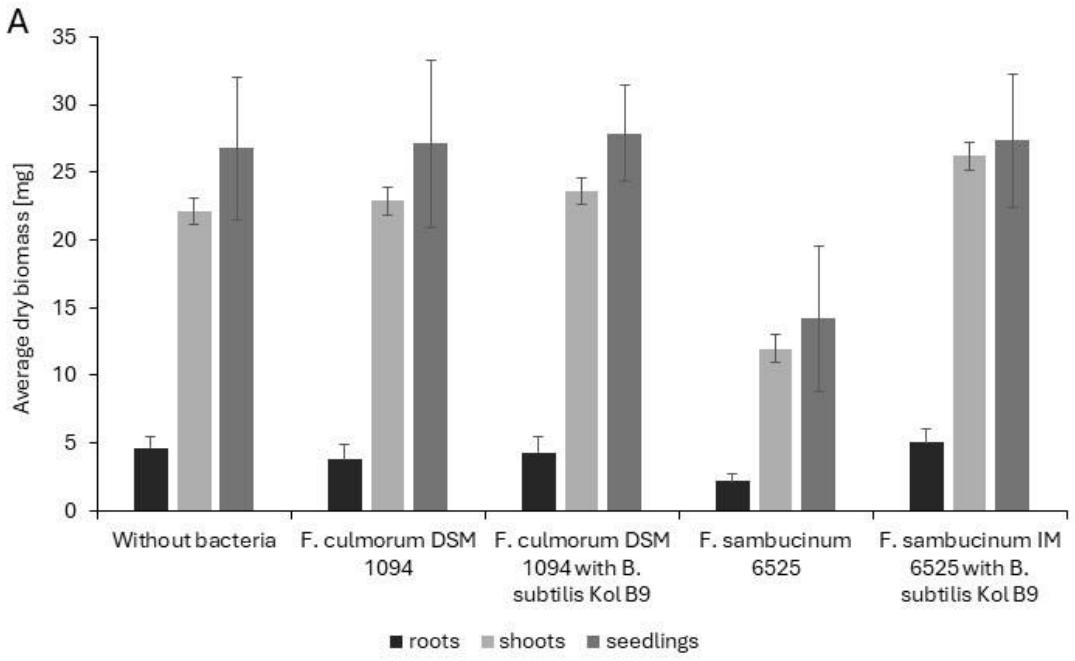


Figure 9. The length (A) and surface area (B) of seedlings hypocotyls grown from seeds inoculated in distilled water (control) or of *B. subtilis* Kol B9.

The data on hypocotyl length and surface area indicate that inoculation with *B. subtilis* Kol B9 significantly stimulated the growth of the above-ground parts of cucumber seedlings (Fig. 9). Seed treatment with Kol B9 bacteria resulted in a 24% increase in hypocotyl length and a 35% increase in hypocotyl surface area compared to the control.

3.5. *In vivo* biocontrol assessment

To determine the biocontrol potential of *B. subtilis* Kol B9, cucumber seedlings were challenged with a *Fusarium* spore suspension in a pot experiment. The pathogen was introduced to the soil as a spore suspension, and cucumber seeds pre-incubated with *B. subtilis* sp. Kol B9 cells were placed in the soil. It was found that cucumber seeds pre-incubated in *Bacillus* suspension were more resistant to the pathogenic *Fusarium* strains (Fig. 10). This effect was the most visible for seedlings treated with *F. sambucinum* IM 6525 spore suspension. The average dry biomass of roots, shoots and whole seedlings obtained from *B. subtilis* Kol B9 treated samples was approximately half that of the control systems where seeds were not pre-incubated in the bacterial suspension.



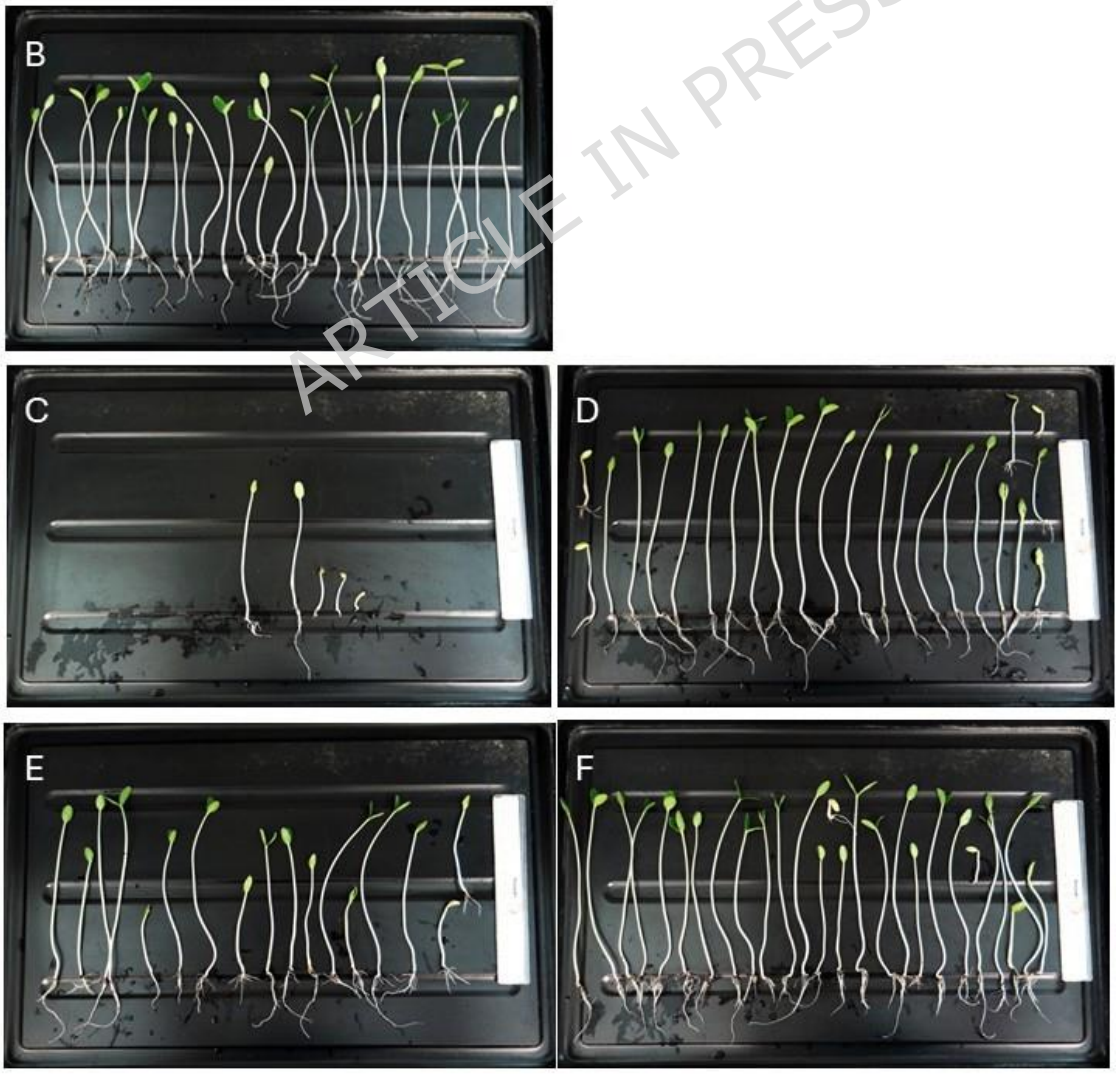


Figure 10. Effect of pre-incubation of cucumber seeds in an aqueous suspension of *B. subtilis* Kol B9 cells on the roots, shoots and whole cucumber seedlings dry biomass (A) in soil with or without *F. culmorum* DSM 1094 or *F. sambucinum* IM 6525 spores. Pictures presents cucumber seedlings obtained from controls (not pre-incubated in *B. subtilis* Kol B9 cells suspension) (B, C, E) and pre-incubated in bacteria cells (D, F), without (B) or with *F. culmorum* DSM 1094 (E, F) or *F. sambucinum* IM 6525 (C, D) spores presence. Error bars represent standard deviation (SD).

Discussion

Biosurfactants production and phytopathogens controlling is a promising feature of *Bacillus* species in the face of climate changes, environmental pollution, and the urgent need for sustainable agricultural solutions. Biosurfactants, with their antifungal properties, could be a natural, eco-friendly alternative to chemical pesticides. Their use may help increase crop yields and protect plants from biotic and abiotic stress. However, in order to optimally use the potential of *Bacillus* bacteria and their metabolites, it is crucial to understand these natural mechanisms. The antagonistic activity of bacteria in relation to the phytopathogenic fungi may be attributed to the composition of the metabolites they produce [26]. Thus, a qualitative analysis of the produced biosurfactant compounds was performed, and the content of surfactin and iturin was determined. Additionally, the MALDI-TOF/TOF method was used to assess the production of fengycin. The strains tested varied significantly in terms of concentration and relative proportions of the lipopeptides produced. The highest surfactin content in post-culture liquids was found for strains Kol B2, Kol D3 and Kol D8, while iturin production peaked in strains Kol B2, Kol B9, Kol D8, Kol Si 4 and Kol Si6. Only strain Kol Si6 did not produce fengycin.

There are relatively few studies investigating the simultaneous production of surfactin, iturin and fengycin by *Bacillus* bacteria. In work by Jasim et al. [27], PCR and LC–MS/MS were used to confirm that the endophytic strain *Bacillus* sp. BmB 9 is capable of the simultaneous production of surfactin, iturin and fengycin. Similarly, Paraszkiewicz et al. [9] identified that three out of six *Bacillus* strains tested produced surfactin, iturin, and fengycin using LC–MS/MS and MALDI–TOF/TOF techniques. As summarized by Crouzet et al. [28], *Bacillus* strains producing surfactin, iturin and fengycin are well-known for their direct antagonism against diverse phytopathogens in different host plants.

In the presented study, the antifungal activity of the tested *Bacillus* strains against *Fusarium* strains was assessed, using both solid and liquid culture systems. The results revealed varied antifungal activity of the tested isolates, with all strains inhibiting the growth of both *Fusarium* strains in both culture types (solid and liquid). However, *F. sambucinum* IM 6525 was more resistant to bacterial inhibition. This difference may be attributed to the unique conditions of a given strain and to the fact that *F. sambucinum* IM 6525 is an environmental strain that was isolated from the rhizosphere of infected raspberry. Environmental strains constantly coexist with bacteria in soil and are exposed to their metabolites. As a result of selection pressure, they can develop defense mechanisms (e.g. production of enzymes that degrade bacterial metabolites, changes in the structure of the cell wall or cell membrane, active pump systems removing antagonistic metabolites of bacteria) build up mechanisms of cooperation with

bacteria and show greater genetic diversity compared to collection strains. This enhances the practical relevance of our findings. In the case of dual culture of the tested *Bacillus* strains with the mycelium of *F. sambucinum* IM 6525, the colonization of the hyphal surface by bacteria and intensive, dendritic growth of the bacterial population towards the parent bacterial colony were observed. This process effectively prevented further colonization of the substrate surface by hyphae. This phenomenon is an example of microbial competition for the growth environment. Bacteria of many *Bacillus* strains can move in the environment using cilia (swimming motility) or move by sliding motility due to the secretion of biosurfactants [29]. The presence of mycelium in the growth environment may facilitate the movement of bacteria using cilia. The above process allows bacterial cells to move along the hyphae and colonize them [30; 31]. According to Haq et al. [32] fungal hyphae can bind water molecules around their cells and thus support bacterial motility.

When comparing the effects of surfactin and iturin on fungal growth, it was found that surfactin had a weaker inhibitory effect on hyphal growth than iturin. However, the antifungal activity is not determined by the concentration of each lipopeptide alone. The proportions of surfactin, iturin, and fengycin are also critical in the interaction between *Bacillus* and pathogenic fungi, including *Fusarium*, which possesses resistant cell wall and defense mechanisms. Different ratios of cLP-Bc may influence fungal growth and development, with iturin and fengycin playing key roles in inhibiting *Fusarium* by disrupting its membranes and preventing spore germination [12, 33]. Surfactin might enhance the antifungal activity of the other two compounds by increasing membrane permeability. Moreover, surfactin also promotes the spread of the bacteria on the surface, which may also indirectly work in favor of antifungal activity. CLP-Bc may also act synergistically - mutually enhance and support each other's antifungal activity. For example, Wang et al. [20] found that surfactin and fengycin produced by the *B. pumilus* W-7 strain showed the ability to inhibit the growth of the *Phytophthora infestans* W101 synergistically. Their study showed, that surfactin is involved in the induction of ISR, while fengycin is primarily responsible for the deformation of fungal cells, the induction of oxidative stress, and the destabilization of mitochondrial function. In this study, among all of the tested bacterial strains, only strains B9 and D3 demonstrated an increase in iturin content (by 10 and 20%, respectively) in bacterial-fungal co-cultures compared to control cultures grown without fungus. Available literature data indicates that strains belonging to species such as *B. amyloliquefaciens, B. velezensis* or *B. subtilis* may respond differently to the presence of antagonistic fungi [34]. For example, Cawoy et al. [35] found that cells of the *B. subtilis* 98S strain significantly increased the production of iturin and fengycin in response to *Pythium*

aphanidermatum and *F. oxysporum* mycelium. However, this effect was not observed in systems containing *Botrytis cinerea* mycelium. Similarly, three strains of *B. velezensis* (SQR9, FZB42, S499) have been shown to respond differently to different strains of fungi by changing their metabolism, leading to specific overproduction of selected cLPs [36, 37]. Both Cawoy et al. [35] and Kulimushi et al. [38] suggested that these changes in the level of cLP production may be bacteria's response to molecular signals released by fungi. It is possible that the bacteria initially produce high levels of surfactin and iturin, but during mycelial growth restriction, these compounds are consumed, which leads to a decrease in their levels in the samples. Another explanation could be a defensive reaction of the mycelium to the presence of biosurfactants, consisting for example of the secretion of molecular signals that modify the production of cLPs, or the production of enzymes involved in their degradation.

To further investigate the cellular response of *Fusarium* to bacterial metabolites, the effects of *Bacillus* sp. B9 were studied on fungal spore germination, hyphal growth, membrane permeability, and lipid content. In the presence of *Bacillus* sp. Kol B9, spore germination was reduced to 60% for *F. sambucinum* IM 6525 and 42% for *F. culmorum* DSM 1094. A similar effect was observed for hyphae length. The lower sensitivity of the environmental *F. sambucinum* isolate may reflect its adaptation to competitive soil environments, where prolonged exposure to diverse microbial communities and antimicrobial metabolites can promote increased tolerance. Consistent with these observations, Hirozawa et al. [39] reported that the post-culture liquids of two *B. subtilis* strains (NT1 and NT2) inhibited the germination of *F. verticillioides* spores (by 22-74%). Zhao et al. [40] also showed that the post-culture liquid obtained from the culture of *B. velezensis* A4 strain effectively inhibited spore germination, hyphal elongation and mycelium growth of *B. cinerea* strain. It was found that the tested post-culture liquid significantly limited the expression of genes of the *B. cinerea* strain related to mycelium growth and phytopathogenicity and limited the ability of spores to penetrate plant cell walls. Liu et al. [41]evaluated the ability of cLPs produced by *B. subtilis* CU12 to limit the germination of spores of phytopathogenic strains belonging to the species *Alternaria solani, F. sambucinum, Rhizopus stolonifer* and *Verticillium dahliae*. The effectiveness of inhibiting spore germination was diverse and depended on the fungus used in the study and on the type of lipopeptide. The authors found that the inhibition of spore germination is closely related to the permeability of cell membrane. It is in agreement with the presented study of *Bacillus* sp. Kol B9 influence on the permeability of *Fusarium* cell membrane. Literature data indicates that the sensitivity of mycelium to cLPs-Bc depends on the lipidome composition. The high sensitivity of fungi to individual biosurfactants may be related to low ergosterol content, the presence of

short-chain phospholipids, low PE level in cell membrane or low degree of fatty acid saturation in these phospholipids [12, 42]. In the present study, by analyzing the percentage content of individual phospholipids in the samples of both mycelia, it was found that the mycelium membranes of *F. culmorum* DSM 1094 contained slightly more fatty acids with a lower degree of saturation than the mycelium membranes of *F. sambucinum* IM 6525. The result indicates a higher susceptibility of the lipidome of *F. culmorum* DSM 1094 mycelium to the action of biosurfactants. In turn, the mycelium membranes of *F. sambucinum* IM 6525 had a lower percentage of PE in relation to PC than the mycelium membranes of *F. culmorum* DSM 1094. This result indicates a higher sensitivity of *F. sambucinum* IM 6525 membranes to the action of biosurfactants. The content of PE and PC in cell membrane may also affect the susceptibility to bacterial biosurfactants. These phospholipids are responsible for the stability, flexibility and fluidity of the lipid bilayer, ensuring its integrity and proper functioning. In this work, *F. culmorum* DSM 1094 membranes contained 8% more PE than *F. sambucinum* IM 6525, suggesting greater permeability to bacterial lipopeptides and therefore greater susceptibility to their adverse effects on the cells. This is reflected in the condition of the membranes assessed after incubation with bacterial cells. The permeability of *F. culmorum* DSM 1094 membranes increased significantly compared to the *F. sambucinum* IM 6525, which corresponds with the results regarding the intensity of mycelium growth in the presence of the bacteria.

Rhizobacteria present in the plant growth environment can lead to an increase in the number of roots, stimulation of lateral root development, or induction of root hair growth and elongation [43-47]. The positive effect of bacteria on roots may result from the production of auxins or other metabolites and is reflected in the development of the whole plant [48, 49]. Cucumber roots growing in the presence of *B. subtilis* Kol B9 bacteria were characterized by increased length, surface area, volume and the number of root tips. This enhancement in root development was also reflected in the increased growth of the above-ground parts of the plant and improved resistance to phytopathogens, particularly *F. sambucinum* IM 6525. Duan et al. [50] reported that *B. amyloliquefaciens* QSB-6 significantly boosted the growth of *Malus hupehensis* seedlings by increasing root length, surface area, and the number of root tips and forks. It also enhanced respiration rates and protective enzyme activities while promoting beneficial soil bacteria and suppressing soil fungi. The fermentation broth from strain QSB-6 also effectively prevented root damage from *Fusarium*. *Bacillus* biopreparations might be applied in the form of solid or liquid fertilizers, and they have shown a good control effect on crop diseases [51] . However, in our study, seeds pre-incubation in bacterial cell suspension has been conducted in growth promoting and antifungal *in vivo* analysis, making the method more practical.

Integration of the lipidomic, permeability, and developmental analyses suggests that different cyclic lipopeptide families contribute to distinct aspects of the antifungal response. Surfactin-type lipopeptides were mainly associated with membrane-related effects, whereas iturin and fengycin families were more closely linked to spore germination inhibition and hyphal growth impairment. This highlights the multifactorial nature of Bacillus-derived lipopeptide activity against *Fusarium* spp.

Conclusions

The results of the presented study reveal that *Bacillus* spp. strains, isolated from non-urban rhizospheric soil,, exhibit multifaceted biological activity, including the production of biosurfactants, antagonism against *Fusarium* spp., and cucumber seedlings growth-promoting properties. Among the tested strains, *B. subtilis* Kol B9 exhibited the highest and most consistent efficacy, in both controlled conditions and in soil-based experiments.

The antagonistic activity of *B. subtilis* Kol B9 was evaluated not only against a model strain (*F. culmorum* DSM 1094), but also against an environmental isolate of *F. sambucinum* IM 6525 originating from rhizospheric soil. Using a rhizospheric *Fusarium* isolate provides insight into biologically relevant interactions, where pathogen adaptability often limits biocontrol effectiveness.

Mechanistic analyses demonstrated that metabolites produced by B. subtilis Kol B9 were associated with reduced spore germination, disruption of hyphal development, and alterations in fungal membrane integrity, reflected by changes in lipid composition and membrane permeability. These effects were particularly evident in *F. culmorum*, which exhibited significant reduction in key phospholipid classes and decreased membrane integrity.

In parallel, *B. subtilis* Kol B9 significantly enhanced cucumber seedling development, with a pronounced effect on root system architecture and mitigation of pathogen-induced stress.. Collectively, these findings highlight the dual functionality of *B. subtilis* Kol B9 as both a biocontrol agent and a plant growth promoter and support the applicability of Kol B9-based bioformulations under realistic, microbially dynamic soil conditions.

In this context, the multifunctional properties of cyclic lipopeptides produced by Kol B9 constitute a key advantage over conventional antimicrobial compounds, combining antifungal activity with ecological compatibility and additional roles in plant–microbe interactions.

Funding

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Data Availability

The 16S rRNA sequence of *Bacillus* sp. Kol B9 has been deposited in GenBank (NCBI) under accession number PX963729 and is publicly available at https://www.ncbi.nlm.nih.gov/nuccore/PX963729. Other datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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