Surface microbiota of *Pinus sylvestris* seeds and its potential in biocontrol

Viacheslav Shopinskyi[1], Liudmyla Butsenko[1,2]

1 - National University of Food Technologies, Kyiv, Ukraine
2 - D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine

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Corresponding author:

Viacheslav Shopinskyi
E-mail:
chemslava@gmail.com

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Abstract

Introduction. The study of plant-associated microbiota deepens our understanding of plant–microorganism interactions and is of considerable practical importance for the identification of strains suitable for the development of biotechnological preparations for crop production. This paper presents the results of an investigation into the epiphytic microbiota of Scots pine (*Pinus sylvestris* L.) seeds and its antagonistic potential.

Materials and methods. Epiphytic bacteria were isolated by washing seeds to preserve native surface communities. The isolates were characterized by morphological and cultural traits and identified using classical microbiological methods and MALDI-TOF mass spectrometry. Antagonistic activity was evaluated in vitro by the radial streak method against phytopathogenic bacteria (*Pseudomonas syringae* 8511, *P. fluorescens* 8573, *Xanthomonas campestris* 8003b, *Agrobacterium tumefaciens* 8628, and *Clavibacter michiganensis* subsp. *michiganensis* 10_2).

Results and discussion. It was found that the epiphytic bacterial microbiota is represented by three species: *Bacillus subtilis*/*B. amyloliquefaciens*, *Pantoea agglomerans* and *Micrococcus luteus*. Epiphytic bacterial isolates from Scots pine seeds were successfully identified using MALDI-TOF mass spectrometry with a high degree of reliability: 99.9%. Structural analysis of the epiphytic microbiome shows an even distribution between two taxa: *B. subtilis*/*B. amyloliquefaciens* and *P. agglomerans*, each of which accounts for 40% of the total number of identified isolates; one fifth of the microbial community (20%) is composed of *M. luteus.*

Bacillus subtilis/*amyloliquefaciens* strain E18 showed the highest antagonistic activity, forming growth inhibition zones of up to 35±1 mm against *X. campestris* and 15±2 mm against *C. michiganensis*. Isolate E15 showed selective activity against *P. syringae* (18±1 mm) and *C. michiganensis* (12±1 mm). Meanwhile, strains E17 and E20 did not inhibit the growth of any of the tested phytopathogens.

Thus, the results of the study confirmed the presence of bacteria with pronounced biocontrol potential among the epiphytic microbiota of *P. sylvestris* seeds. *Bacillus* spp. strains are particularly promising, as they are capable of producing a wide range of biologically active metabolites and demonstrating stable antagonistic activity.

Conclusions. The experimental data obtained provide a basis for the development of environmentally safe biological products that can be used in forestry to increase seed germination and protect seedlings from phytopathogens.

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Introduction

Food safety is a priority area of research, with one of its key components being the achievement of stable crop yields and effective protection against plant pathogens. The plant microbiome plays a crucial role in maintaining plant health by contributing to pathogen suppression, growth stimulation, and tolerance to abiotic stresses. These microbial communities, inhabiting internal tissues (endophytes) and plant surfaces (epiphytes), are an integral part of plant life (Lindow et al., 2003; Turner et al., 2013).

Epiphytic bacteria are microorganisms that colonize the external surfaces of plants, including leaves, stems, flowers, and seeds, without penetrating host tissues. Unlike endophytic microorganisms, epiphytic microbiota form complex microbial communities on plant surfaces, collectively referred to as the phyllosphere (Lindow et al., 2003; Vorholt, 2012).

Among epiphytic bacteria, representatives of the genera *Pseudomonas, Bacillus, Pantoea, Sphingomonas, Methylobacterium*, and *Enterobacter* are the most common. These microorganisms exhibit antagonistic activity against phytopathogens and are capable of producing a wide range of biologically active substances, including antibiotics, siderophores, phytohormones, terpenes, and other metabolites that enhance plant resistance to biotic and abiotic stress factors (Achath et al., 2025; Gnanamanickam et al., 2006; Hirano et al., 2000; Lindow et al., 2003; Vorholt, 2012; Trias et al., 2012).

The microbiota associated with seeds is important and largely derived from rhizosphere microbial communities, although partial vertical transmission from the host plant has also been reported. These microorganisms can be beneficial, promoting seedling germination and development, or pathogenic, leading to seed degradation and damage to the host plant, depending on the species composition and functional characteristics of the microorganisms (Necajeva et al., 2023).

Scots pine (*Pinus sylvestris* L.) plays a key role in the formation of coniferous forest ecosystems in northern Europe. The cultivation of this species faces specific challenges, particularly infection of young plants by phytopathogenic fungi, such as *Fusarium* spp., which cause fusarium wilt and severely affect the germination capacity of seed material. Additional problems arise from adverse abiotic conditions, including temperature extremes, salt stress, and heavy metal exposure. The combined impact of these biotic and abiotic factors leads to reduced germination energy, lower percentages of viable seedlings, and slowed ontogenetic development of the young tree generation (Pirttila et al., 2011).

Conventional phytoprotection methods based on the use of chemical fungicides, bactericides and synthetic pesticides have limited effectiveness and are characterised by negative ecotoxicological potential, causing contamination of natural environments and inducing the formation of resistant strains of phytopathogenic microorganisms (Pal et al., 2006; Pirttila et al., 2011). Therefore, the search for environmentally safe alternatives based on the use of antagonistically active epiphytic bacteria is a relevant and promising area of research.

Although endophytic bacteria from Scots pine seeds have been extensively studied for their growth-stimulating and protective effects, the epiphytic bacterial community found directly on the surface of these seeds remains less studied. Due to their surface colonisation, epiphytic bacteria are the first line of microbial interaction with germinating seeds and young seedlings, potentially providing protective and growth-stimulating benefits. The study of epiphytes on seeds is extremely important for understanding the initial points of interaction and their response to external environmental factors. These microorganisms, which are found on the surface of the seed, are the first to interact with external stress factors during germination: moisture, temperature, ultraviolet radiation, and potential pathogens (e.g., soil fungi, airborne bacteria). The study of epiphytic bacteria is important for understanding the early interactions between plants and

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microorganisms and for developing targeted seed treatment methods that provide effective phytostimulant effects (Aachath et al., 2025; Gnanamanickam et al., 2006).

The plant microbiome has a significant impact on the health of the host plant, its growth and resistance to biotic and abiotic stresses. These microbial communities, which include bacteria and fungi, interact with plants in complex and dynamic ways, shaping their development and adaptation to stress factors (Turner et al., 2013). Epiphytic bacteria are a major component of the diverse microbiota that inhabits the surface of plants. They live predominantly non-parasitically on various plant organs, including seeds, forming aggregates or biofilms (Gnanamanickam et al., 2006; Thomas et al., 2024).

Epiphytic bacteria have significant potential in sustainable agriculture due to their ability to promote plant growth. This is often achieved through the production of phytohormones (auxins, cytokinins, and gibberellins), which are important for plant development. For example, some *Bacillus* species are known to produce gibberellins (Aachath et al., 2025; Turner et al., 2013). They can also solubilise essential nutrients (zinc, potassium, phosphorus), making them bioavailable to plants. Some bacterial species are also capable of fixing atmospheric nitrogen, providing additional symbiotic benefits (Aachath et al., 2025).

Epiphytic bacteria can effectively suppress phytopathogens through various mechanisms. Key mechanisms include competitive exclusion, where beneficial microbes outcompete pathogens for essential nutrients (carbon, nitrogen, hydrogen, oxygen, sulphur, phosphorus) and space on the plant surface. In addition, many epiphytic species secrete antimicrobial compounds (e.g., antiviral, antibacterial, and antifungal bios compounds) that directly inhibit pathogen growth (Gnanamanickam and Immanuel, 2006).

Studies of the *Pinus sylvestris* microbiome have mostly focused on endophytic communities, especially in the roots. These studies have shown that factors such as geographical origin and season significantly influence the composition and nature of interactions between root endophytic fungal and bacterial communities, correlating with the biochemical properties of the host plant's roots and climatic factors (Maitra et al., 2024).

In the context of studying the epiphytic microbiota of Scots pine (*Pinus sylvestris* L.) seeds, it is important to consider the specific conditions of these microorganisms. The surface of conifer seeds is characterised by low moisture content, the presence of resinous substances and other secondary plant metabolites, which creates selective pressure for the formation of a specific microbial community (Pirttila et al., 2011).

Particular attention in studies is paid to epiphytic bacteria of the genus *Bacillus*, which demonstrate high efficiency as biological control agents and are characterised by their ability to produce various antimicrobial compounds, lipopeptides and surface-active substances (Aachath et al., 2025; Monteiro et al., 2005).

Studies have also shown that epiphytic bacteria *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus cereus*, isolated from cabbage and radishes, exhibit significant antagonistic activity against the phytopathogen *Xanthomonas campestris*. The authors noted that inoculation of plants with epiphytic bacteria contributed to increased germination and reduced pathogen infection of seedlings (Monteiro et al., 2005).

Scientists have established that the microbiota of seeds of plants of the *Brassicaceae* family contains endophytic and epiphytic microorganisms of the genera *Bacillus*, *Massilia*, *Pseudomonas* and *Pantoea*, which exhibit growth-stimulating activity on the germination and initial development of the host plant (Barret et al., 2015).

An important aspect is the study of factors that influence the formation of epiphytic seed microbiota. According to scientific research, the composition of the microbiota depends on the plant genotype, environmental conditions, seed storage methods and other factors (Compant et al., 2019).

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In the field of forestry, the study of conifer seed microbiota is particularly relevant. The authors note that conifer seeds are often characterised by low germination and high sensitivity to pathogens, which makes the search for natural antagonists among the microbiota a promising area of research (Pirttila et al., 2011).

Fusarium filamentous ascomycete fungi are well-known pathogens of *Pinus sylvestris* seeds, capable of significantly reducing germination rates (Davydenko, 2019; Davydenko et al., 2018; Yusipovich et al., 2018). This highlights the need for effective biological control strategies. Despite active research into phytomicrobiome interactions and recognition of the importance of seed microbial associations, there is insufficient study of the epiphytic bacterial community of *Pinus sylvestris* seeds and its functional characteristics. Therefore, comprehensive characterisation, study of the species diversity of epiphytic bacteria in pine seeds, and experimental determination of their antagonistic activity against phytopathogenic microorganisms is a relevant scientific task that can contribute to the development of environmentally safe methods for protecting and stimulating the growth of this important forest species.

The aim of the present work is to study epiphytic bacteria isolated from the seed material of Scots pine (*Pinus sylvestris* L.); to establish the taxonomic affiliation of surface microflora, characterise its morphological and cultural features, and assess the bioantagonistic potential of identified epiphytic isolates against phytopathogenic microorganisms.

Materials and methods

Materials

For the study, first-class Scots pine (*Pinus sylvestris* L.) seeds harvested in 2023 from the Bucha Forestry Enterprise (Kyiv region) were used. The seed material was stored under optimal conditions to preserve its quality until laboratory analysis.

Phytopathogenic bacteria strains

To study the antagonistic activity of epiphytic bacteria isolated from Scots pine (*Pinus sylvestris* L.) seeds, a collection of well-characterised strains of phytopathogenic bacteria was used: *Pseudomonas syringae* 8511; *Pseudomonas fluorescens* 8573; *Xanthomonas campestris* 8003b; *Agrobacterium tumefaciens* 8628; *Clavibacter michiganensis* subsp. *michiganensis* 10_2.

Test cultures of the phytopathogenic bacteria were obtained from the collection of the Department of Phytopathogenic Bacteria of the D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Isolation of epiphytic bacteria

For selective isolation of epiphytic bacteria, a "soft" washing method was used, which ensured the preservation of surface microorganisms without the use of harsh sterilisation procedures characteristic of endophyte isolation. This methodological difference is critical for differentiating between epiphytic and endophytic microbial communities.

A washing method was used to isolate epiphytic bacteria from the surface of Scots pine seeds (Baruco et al., 2020; Golub et al., 2012; Sanchez-Lopez et al., 2018). A seed sample (50 seeds) was placed in 100 ml of sterile 10 mM $MgSO_4$ solution and intensively stirred on a mechanical shaker for 20 minutes at 200 rpm to desorb surface-associated microbial cells

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(Necajeva et al., 2023). The resulting wash solution containing the epiphytic microbial community was thoroughly mixed to ensure uniform distribution of cells and serial tenfold dilutions (10^{-1}, 10^{-2}, 10^{-3}) were prepared. Aliquots of 0.1 ml from the corresponding dilutions were transferred with a sterile pipette to the surface of agarised nutrient media: potato agar (for fungi) and LB medium (for bacteria). The inoculum was evenly distributed over the surface of the medium using a sterile L-shaped spatula. The Petri dishes were labelled, turned upside down and incubated at a temperature of 27 ± 1°C. The development of bacterial colonies was monitored every 24 hours for 72 hours. Morphologically distinct and representative bacterial colonies were selected for further purification by repeated transfer to fresh nutrient media until pure cultures were obtained for further identification and characterisation.

Nature of isolated bacteria

The isolated epiphytic bacterial strains were characterised using classical microbiological methods (Patika et al., 2014; 2017). This included assessment of colony morphology (size, shape, colour, transparency, surface texture, edge characteristics), cell morphology (shape, arrangement), Gram reaction, and endospore presence. Physiological and biochemical tests were performed to determine key metabolic properties: catalase and oxidase activities, ability to grow on media with different NaCl concentrations (2%, 5%, 7%, 10%), aerobic and anaerobic growth on glucose; hydrolysis of gelatin, starch, casein and esculin. Nitrate reduction, tyrosine hydrolysis, citrate utilisation and the Voges-Proskauer reaction (acetoine production) were also evaluated. The optimal, minimum and maximum temperatures and pH ranges for growth were determined.

Use of MALDI-TOF-MS for the identification of epiphytic bacteria

Taxonomic identification of purified epiphytic bacterial isolates was performed using matrix-assisted laser desorption/ionisation (MALDI-TOF) mass spectrometry using the VITEK MS system. The study included the preparation of a calibration curve using Escherichia coli ATCC 8739. Pure cultures of test bacteria, grown for 18-24 hours, were applied in a thin layer to the MALDI-TOF target well of the plate. A matrix solution (α-cyano-4-hydroxycinnamic acid) was then added to each target well and allowed to air dry. The prepared slide was placed in the VITEK MS device and the identification process was started. Bacteria identification was achieved by comparing the obtained mass spectra of unknown isolates with an extensive database of reference spectra. The system provides a percentage of identification reliability, with a range from 60% to 100% indicating correct identification (Ashfaq et al., 2022; Cevik et al., 2021; Tsuchida et al., 2020).

Antagonistic activity of epiphytic bacteria

The antagonistic activity of epiphytic bacteria isolated from Scots pine seeds against bacterial phytopathogens was evaluated in vitro using the radial streak method (co-culture test) on appropriate agar media. A streak of the antagonist (epiphytic isolate) was made on an agar plate, and then streaks of phytopathogens were made perpendicular to the isolates. The plates were incubated at 27°C for 24-48 hours. Antagonistic activity was quantitatively assessed by measuring the growth inhibition zone (in millimetres) of the phytopathogen around the antagonist streak (Butsenko et al., 2010; Patyk et al., 2017).

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Assessment of experimental results

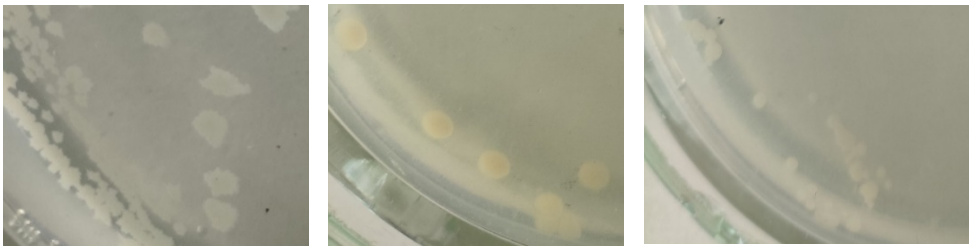
Experimental studies were conducted with three replicates for each variant. Mathematical and statistical analysis of the obtained data was performed using the Statistica 6.0 software package with standard Microsoft Excel statistical tools.

Results and discussion

Isolation and characterisation of epiphytic bacteria from Scots pine seeds

During the study of the epiphytic microbiota of Scots pine (*Pinus sylvestris* L.) seeds, bacterial isolates were isolated and characterised from the seed surface without sterilisation. The method used effectively isolated microorganisms associated with the seed surface and allowed the natural epiphytic microbiota of pine seeds to be preserved for further study of its species composition and functional properties.

Bacteria forming colonies of various morphologies were isolated from the surface of Scots pine seeds. Significant variability in the morphological and cultural characteristics of the isolated isolates (their size, shape, transparency, surface texture, and others) was observed, indicating a high biodiversity of the epiphytic microbiota of the seeds. For detailed study and characterisation, the most typical isolates demonstrating various morphological and cultural characteristics were selected: E13, E16, E17, E18 and E20 (Figure 1).



a **b** **c**

Figure 1. Colonies of epiphytic bacteria from Scots pine seeds: isolate E15 (a), isolate E16 (b), and isolate E17 (c)

Morphological and cultural analysis showed that among the isolated isolates (E15, E16, E17, E18, E20), Gram-positive rods predominate, which are capable of growing on various nutrient media under aerobic conditions. most isolates showed a positive reaction to the catalase test and the ability to hydrolyse gelatin (Table 1).

As shown in Table 1, various morphological, cultural, physiological, and biochemical properties were found among the isolated epiphytes. Isolates E15 and E18, identified as *B. subtilis* (or *B. amyloliquefaciens*), formed rounded or irregular colonies with a diameter of 2-4 mm, with wavy or fringed edges, opaque, with a wrinkled surface, whitish in colour. They are Gram-positive, motile, straight rods that form ellipsoidal and cylindrical spores. These isolates showed positive results for catalase activity, hydrolysis of gelatin, starch, casein and esculin, as well as the ability to reduce nitrates and utilise citrate. They demonstrated growth at NaCl concentrations up to 10% and aerobic growth on glucose, with an optimal growth temperature of 28-30 °C and a pH range of 5.5-8.5.

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Table 1

Properties of epiphytic isolates isolated from Scots pine (*Pinus sylvestris* L.) seeds

Property	Isolates from Scots pine seeds			Properties of typical representatives of species according to literature data			
	E15, E18	E16, E20	E17	*B. subtilis*[1]	*B. amyloliquefaciens*[2]	*P. agglomerans*[3]	*M. luteus*[4]
Cell shape	Rods	Rods	Cocci	Rods	Rods	Rods	Cocci
Spore formation	+	-	-	+	+	-	-
Gram staining	+	-	+	+	+	-	+/-
Mobility	-	+	-	+	+	+	-
Catalase activity	+	+	+	+	+	+	+
Oxidase activity	+	-	+	+/-	-	-	+
Growth in a medium with 2% NaCl	+	+	+	+	+	+	+
Growth in a medium with 7% NaCl	+	-	-	+	+	+/-	-
Growth in a medium with 10% NaCl	+	-	-	+/-	+/-	N/A	-
Growth in glucose medium aerobically	+	+	+	+	+	+	+
Growth in glucose medium anaerobically	-	+	-	+/-	+/-	+/-	-
Gelatin hydrolysis	+	+	-	+	+	+	-
Starch hydrolysis	+	-	-	+	+	-	-
Casein hydrolysis	+	-	-	+	+	N/A	+
Esculin hydrolysis	+	+	-	+	+	+/-	-
Nitrate reduction	+	+	-	+	+	+	-
Citrate utilization	+	+	-	+	+	+	-
Voges-Proskauer reaction	+\-	+	-	+	+	+	+/-

Note: N/A – not found; 1 – according to Logan et al., 2015; Rooney et al., 2009; Satapute et al., 2012; 2 – according to Baudu et al., 2025; Afrin et al., 2023; 3 – according to Mardaneh et al., 2013; Acioly et al., 2017; 4 – according to Shi et al., 2023; Greenblatt et al., 2004; Environment and Climate Change Canada, 2018.

Isolates E16 and E20, identified as *Pantoea agglomerans*, formed smooth, translucent, convex colonies with intact edges and a pale yellowish colour. They are Gram-negative, motile, straight rods that do not form spores. They are characterised by catalase activity,

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gelatin hydrolysis, nitrate reduction and citrate utilisation. They are capable of growing at 7% NaCl, with an optimal temperature of 25-30 °C and a pH range of 4.5–9.0.

Isolate E17, identified as *Micrococcus luteus*, formed round, smooth, creamy-yellow colonies approximately 4 mm in diameter. The cells are coccoid, Gram-positive, immotile, and do not form spores. This isolate exhibited catalase and oxidase activity, casein hydrolysis, but did not hydrolyse gelatin and starch. Growth was observed at 5% NaCl. The optimal growth temperature was 25–30 °C and the pH range was 7.0–9.0.

This characterization confirmed the greater taxonomic and functional diversity of the epiphytic community compared with the predominantly bacillary endophytic community described by Shopinskyi and Butsenko (2025).

MALDI-TOF MS-based identification of epiphytic bacteria

The identification of epiphytic bacteria was also carried out using the MALDI-TOF mass spectrometry method, which ensured high accuracy of taxonomic determination.

Taxonomic identification of the studied bacterial isolates was performed using the matrix-assisted laser desorption/ionisation mass spectrometry method.

Based on the results of MALDI-TOF mass spectrometric analysis, the taxonomic affiliation of five epiphytic bacterial strains (E15, E16, E17, E18 and E20) isolated from the surface of *Pinus sylvestris* seeds was established. The dominant taxa were representatives of *B. subtilis*/*B. amyloliquefaciens* and *P. agglomerans*. A single isolate of *M. luteus* was also identified.

Five separate epiphytic bacterial isolates (E15, E16, E17, E18, E20) were successfully identified using MALDI-TOF mass spectrometry with a high degree of reliability: 99.9% (Table 2).

Table 2

Results of identification of epiphytic bacterial isolates using MALDI-TOF

Isolate	Identified epiphytic bacteria	Identification accuracy, %
E15	*Bacillus subtilis/amyloliquefaciens/vallismortis*	99.9
E16	*Pantoea agglomerans*	99.9
E17	*Micrococcus luteus*	99.9
E18	*Bacillus subtilis/amyloliquefaciens/vallismortis*	99.9
E20	*Pantoea agglomerans*	99.9

Diversity of the epiphytic microbiota of Scots pine seeds

The epiphytic bacterial microbiota of *Pinus sylvestris* L. seeds is characterised by limited taxonomic diversity and is represented by three dominant species: *B. subtilis*/ *B. amyloliquefaciens* (for isolates E15 and E18), *P. agglomerans* (for E16 and E20) and *M. luteus* (for E17).

Structural analysis of the epiphytic microbiome shows an even distribution between two taxa: *B. subtilis*/*B. amyloliquefaciens* and *P. agglomerans*, each of which accounts for 40% of the total number of identified isolates; one fifth of the microbial community (20%) is composed of *M. luteus* (Figure 4).

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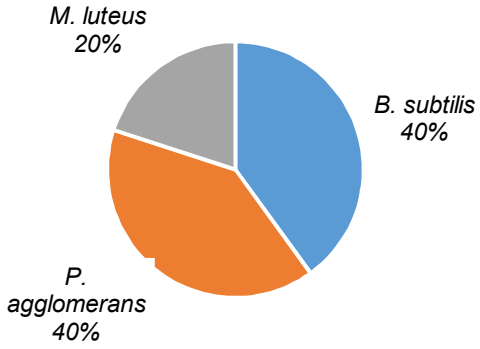

Figure 4. Species distribution of epiphytic bacteria isolated from Scots pine (*Pinus sylvestris* L.) seeds

The identified structure of the epiphytic microbiome reflects the selective adaptation of bacterial taxa to the specific ecological conditions of the surface of conifer seeds. The dominance of *Bacillus* spp. reflects their high resistance to stress factors and ability to form spores, which ensures survival in conditions of variable humidity and temperature fluctuations. The presence of *P. agglomerans* may be associated with their phytostimulating properties and ability to colonise plant surfaces, while *M. luteus* is characterised by high resistance to UV radiation and water deficiency.

Biocontrol potential of Scots pine epiphytes against phytopathogenic bacteria

The results of determining the antagonistic activity of epiphytic bacterial isolates isolated from *Pinus sylvestris* L. seeds in relation to collection strains of phytopathogenic microorganisms are presented in Table 3.

Table 3

Antagonistic activity of epiphytic bacteria isolated from *Pinus sylvestris* L. seeds against phytopathogenic bacteria

Epiphytic bacteria	***P. syringae* 8511**	***P. fluorescens* 8573**	***X. campestris* 80036**	***A. tumefaciens* 8628**	***C. michiganensis subsp. michiganensis* 10_2**
	Growth inhibition zones, mm				
E15	18±1	17±1*	N/A	5±1	12±1
E16	0	9±1	0	0	0
E17	0	0	0	0	0
E18	14±1	14±1	35±1	12±1	15±2
E20	0	0	0	0	0

Notes: * – no complete absence of growth was observed, only its delay; N/A – not studied

The data obtained indicate significant variability in the antagonistic activity of the studied epiphytes. Isolates E15 and E18 (*B. subtilis*/*B. amyloliquefaciens*) showed antagonistic activity, forming the largest growth inhibition zones against most of the

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collected phytopathogenic strains: *X. campestris* (35±1 mm), *C. michiganensis* subsp. *michiganensis* (15±2 mm), *P. syringae* (14±1 mm) and *P. fluorescens* (14±1 mm). This indicates a broad spectrum of antimicrobial activity of this isolate and its potential as an effective biological control agent.

Strain E15 showed selective antagonistic activity, exhibiting high efficacy only against *P. syringae* (18±1 mm) and moderate activity against *C. michiganensis* subsp. *michiganensis* (12±1 mm). It was also found that strain E15 has moderate bioantagonistic activity against *P. fluorescens* (17±1 mm) and only inhibits the growth of the phytopathogen, but does not completely eliminate it, indicating a bacteriostatic rather than bactericidal effect.

Isolate E16 was characterised by a narrow spectrum of antagonistic activity, exhibiting an inhibitory effect only against *P. fluorescens* (9±1 mm). Such selectivity may indicate a specific mechanism of antimicrobial action or a limited spectrum of antibiotic compounds produced.

Strains E17 and E20 did not show antagonistic activity against any of the tested phytopathogens. This may indicate that these strains are unable to produce antimicrobial compounds that are effective against the studied phytopathogens, or that they have other mechanisms of interaction with the host plant that are not related to direct antagonism.

Compared to endophytic bacteria, epiphytic isolates show more variable antagonistic activity with less characteristic efficacy indicators. However, strain E18 was characterised by a pronounced inhibitory effect on *X. campestris*, where the growth inhibition zone (35±1 mm) exceeded the results obtained for endophytic bacteria.

The results confirmed the potential of epiphytic bacteria isolated from Scots pine seeds as biocontrol agents, especially strains E15 and E18, which may be promising for further study as potential agents of biological control of phytopathogenic bacteria in forestry.

Conclusions

The isolated epiphytic bacteria of Scots pine (*Pinus sylvestris* L.) seeds, identified as *Bacillus subtilis*/*amyloliquefaciens*, *Pantoea agglomerans* and *Micrococcus luteus*, are characterised by significant biocontrol potential for suppressing phytopathogenic microorganisms. The established taxonomic structure of the epiphytic microbiome demonstrates the dominance of bacillary forms and the presence of phytostimulating taxa adapted to the specific ecological conditions of the surface of coniferous seeds. The antagonistic activity of *Bacillus* spp. strains against a wide range of phytopathogens confirms the promise of their use in the creation of environmentally safe biological products for forestry. Further research should be aimed at studying the molecular mechanisms of antagonistic action, identifying antimicrobial metabolites, and evaluating the effectiveness of selected epiphytic isolates in field conditions for protecting seedlings and increasing seed germination.

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Author identifiers ORCID

Viacheslav Shopinskyi 0009-0006-9050-8896

Liudmyla Butsenko 0000-0002-3575-4289

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