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Biocontrol potential of Antarctic endophytic bacteria

Abstract. Antarctic endophytes, adapted to harsh environmental conditions, possess unique metabolic capabilities that can influence plant-microbe interactions. In this study, we investigated the impact of 15 plant growth-promoting bacterial strains isolated from *Deschampsia antarctica* and *Colobanthus quitensis* on the growth of phytopathogenic fungi. Besides everything else, among growth-promoting traits it was shown that bacteria synthesized biosurfactants, ammonia, and auxin-like hormones for plant growth, and also have shown significant growth in a wide temperature range. While these endophytes exhibited significant antifungal activity against agriculturally important fungi, we also observed the stimulation of fungal growth by certain strains. This dual role of endophytes highlights the complex and context-dependent nature of plant-microbe interactions. Our findings suggest that the effects of endophytes on plant health can be multifaceted. While they can directly inhibit pathogens, they can also indirectly influence the plant microbiome, potentially leading to beneficial and detrimental outcomes. Further research is needed to elucidate the mechanisms underlying these complex interactions and to harness Antarctic endophytes' potential for sustainable agriculture.

Keywords: Antarctic region, antifungal activity, plant-microbe interactions

1 Introduction

Plants are constantly exposed to a myriad of biotic and abiotic stresses, including pathogens, herbivores, drought, salinity, and extreme temperatures. To survive and thrive in these challenging conditions, plants have evolved sophisticated strategies, including establishing symbiotic relationships with beneficial microorganisms. Endophytes, microorganisms that colonize plant tissues without causing apparent disease symptoms, play a crucial role in plant health and resilience. Endophytic bacteria, which colonize both intercellular and intracellular spaces within plant tissues by forming biofilms, are ubiquitous in the natural ecosystem, associating with all plants. In terms of biodiversity, endophytic bacteria are among the most diverse species on earth (Mamarasulov et al., 2023). Many studies have shown the antifungal activity of endophytic bacteria associated with different species of plants, including medicinal herbs (Lodewyckx et al., 2002; Santoyo et al., 2016; Duhan et al., 2020). Once inside the plant, endophytes can adopt various lifestyles, ranging from latent to systemic. In return for the protection and nutrient supply provided by the plant, endophytes can confer various benefits to their host. For example, they can enhance plant growth, stress tolerance, and resistance to pathogens and herbivores (Mengistu, 2020).

In recent years, there has been growing interest in understanding the role of endophytes in plant adaptation to harsh environments. Extreme environments, like the Arctic and Antarctic regions, present unique challenges for plant survival. The harsh conditions, including low temperatures, high salinity, and intense UV radiation, limit plant growth and development. However, plants in these regions have developed strategies to cope with these stresses, often with the help of endophytic microorganisms. By understanding the complex interactions between plants, bacteria, and fungi within the plant microbiome, we can develop novel strategies for improving crop productivity and resilience in the face of climate change and other environmental challenges. Endophytes have established intricate symbiotic relationships with their host plants. These associations often involve the production of bioactive compounds by the endophytes, providing the plant with enhanced defense against pathogens (Barra et al., 2016). This ecological interplay highlights the potential of endophytes as natural biocontrol agents, offering a sustainable alternative to synthetic pesticides in modern agriculture (Rong et al., 2020). By colonizing plant tissues, endophytes can protect their hosts from fungal pathogens, suggesting their potential as a sustainable source of natural antifungal compounds. Endophytic bacteria synthesize many biologically active secondary metabolites, including flavonoid, terpenoid, phenolic, and volatile organic compounds. These molecules can effectively inhibit fungal growth (Deshmukh et al., 2018). Within the "endophytic bacteria-host plant" system, bacteria provide first-line defense for keeping plants healthy and protected from invasive pathogenic fungi that could cause plant diseases.

This study aimed to assess the antifungal potential of bacterial strains associated with Antarctic vascular plants in order to define promising sources of novel antifungal agents with further applications in medicine, agriculture, and industry.

2 Materials and methods

2.1 Bacterial strains used in the study

We used 15 bacterial cultures isolated from *Deschampsia antarctica* and *Colobanthus quitensis* samples (Iungin et al., 2024) collected during the 24th Ukrainian Antarctic Expedition (January—April 2020) along the western part of the Antarctic Peninsula. A short description of the studied bacterial cultures is represented in the Table. Bacterial growth was assessed across a temperature range of 4, 26, 37, and 42 °C (72 hours, Nutrient Broth media) by measuring absorbance at OD600 using a UV-Vis spectrophotometer (Ulab, Shanghai, China).

2.2 Antifungal activity studies by the disk-diffusion method

The antifungal activity of bacterial isolates was determined using the agar disk-diffusion method (Elkahoui et al., 2012) against six phytopathogenic fungi (*Nigrospora oryzae* 15966, *Fusarium solani* 50718, *Nectria inventa* 3041, *Botrytis cinerea* 16884, *Sclerotinia sclerotiorum* 16883, and *Rhizoctonia solani* 16036) obtained from the Ukrainian Collection of Microorganisms from the D. K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine. Besides the fact that those fungi are not presented in the Antarctic region, they are used as model test species in bacterial-fungal assays due to their wide host range, ability to grow *in vitro*, and their significant economic impact in various crops (Kushwaha et al., 2020). Five-day-old fungal mycelia were aseptically inoculated with a sterile needle into the center of Petri dishes filled with a nutrient media. Following equidistant inoculation of bacterial cultures, plates were incubated at 25 °C for five days. The degree of fungal growth inhibition was subsequently determined and expressed as a percentage (Iungin et al., 2024).

2.3 Bacterial metabolites assay

Overnight bacterial cultures (Nutrient broth, 25 °C) were used for 100 ml of sterile media inoculation in a 250-mL Erlenmeyer flask. They were incubated for 3 days in a shaker-incubator set to 140 rpm at 25 °C. Bacterial cultures were then centrifuged for 15 minutes at 10 000 rpm at room temperature. The supernatants were sterilized by filtration using 0.22 μm filters and added to melted agar media reaching 20% (w/v). A 5-day mycelium of pre-grown fungi culture was placed in the middle of the prepared Petri dish with a sterile needle. The results were presented as a percentage of fungal growth according to control samples with fungi grown on regular media.

2.4 Indole-3-acetic acid (IAA) production

To assess IAA production, bacterial isolates were grown in nutrient broth supplemented with 0.2% (w/v) L-tryptophan at 26 °C for 72 hours. Following centrifugation (4 000 rpm for 10 min), the supernatant was collected and reacted with Salkowski's reagent. The development of pink color, indicative of IAA production, was measured spectrophotometrically at 535 nm (UV-Vis spectro-

Table. Endophytic bacteria isolated from Antarctic vascular plants

Strain number	Species	Place of isolation	Coordinates	Cultivation temperature range, °C
Host plant *Deschampsia antarctica*				
9.1	*Siminovitchia terrae*	Lahille Island	−65.553580° −64.394883°	15−42
15.6	*Arthrobacter psychrochitiniphilus*	Ronge Island	−64.683430° −62.644170°	15−30
16.7	*Arthrobacter psychrochitiniphilus*	Ronge Island	−64.683430° −62.644170°	15−30
23.2	*Agreia sp.*	Santos Peak, Graham Passage	−64.405750° −61.547410°	15−30
24.3	*Pseudomonas sp.*	Santos Peak	−64.405750° −61.547410°	4−37
24.4	*Pseudomonas yamanorum*	Santos Peak	−64.405750° −61.547410°	15−42
25.2	*Hafnia psychrotolerans*	Galindez Island	−65.244807° −64.255709°	4−37
26.2	*Pseudomonas sp.*	Galindez Island	−65.244807° −64.255709°	4−42
26.6	*Lysinibacillus macroides*	Galindez Island	−65.244807° −64.255709°	4−30
26.7	*Pseudoarthrobacter sp.*	Galindez Island	−65.244807° −64.255709°	4−42
40.1	*Kocuria salsicia*	Lagotellerie Island	−67.88486° −67.38765°	15−42
Host plant *Colobanthus quitensis*				
10.1	*Pseudomonas salomonii*	Lahille Island	−65.553580° −64.394883°	15−42
10.4	*Psychrobacter arcticus*	Lahille Island	−65.553580° −64.394883°	4−37
39.4	*Pseudomonas sp.*	Lagotellerie Island	−67.884860° −67.387650°	4−37
39.12	*Brachybacterium sp.*	Lagotellerie Island	−67.884860° −67.387650°	4−42

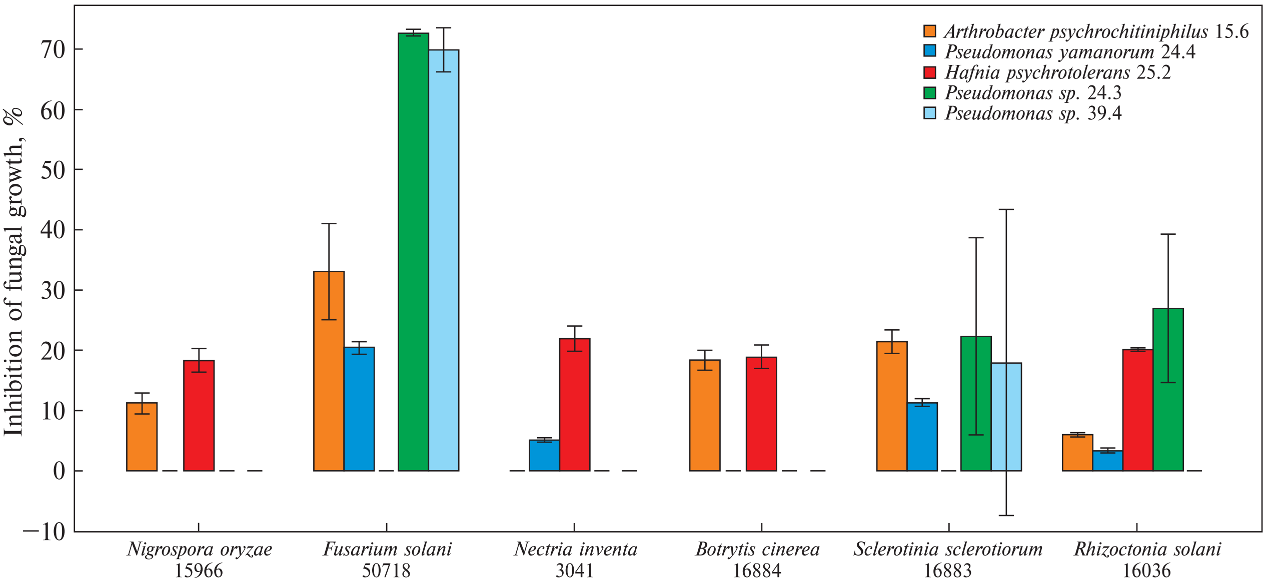

Figure 1. Antifungal activity of the endophytic bacteria (data presented as mean ± SD, %)

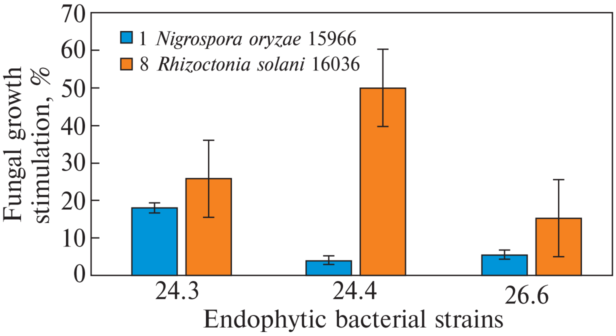

Figure 2. Stimulation of plant pathogenic fungi by endophytic bacteria – *Pseudomonas sp.* 24.3, *Pseudomonas yamanorum* 24.4, and *Lysinibacillus macrolides* 26.6 (fungal growth stimulation is presented as percentage to the control fungal growth area)

photometer Ulab, China). Commercial IAA was used as a standard for comparison.

2.5 Drop collapse assay

A drop collapse assay was performed to assess biosurfactants (BSF) production according to (Thavasi et al., 2011) using a Parafilm hydrophobic film. Reduction of the surface tension and collapse of a 10 μL droplet (an aliquot of bacterial overnight culture) indicated the presence of biosurfactants.

2.6 Ammonia production

Bacterial cultures were grown in peptone water at 26 °C for 48 hours to assess ammonia production. After incubation, Nessler's reagent was added to the supernatant. A change to yellow-brown was indicative of ammonia production (Abdelwahed et al., 2022).

2.7 Statistical analyses

Experimental replicates (n) were used, and quantitative results were presented using bar plots with the median and standard deviation (SD). We also compared multiple groups using a one-way analysis of variance (ANOVA) using Python (visual studio code) with a Tukey post hoc test. For all tests, $p < 0.05$ was considered significant. However, the results were inconclusive, so we do not present the data here.

3 Results

3.1 Antifungal activity

The extreme Antarctic environment, with its low temperatures, high UV radiation, and limited water resources, shapes the habitat of vascular plants

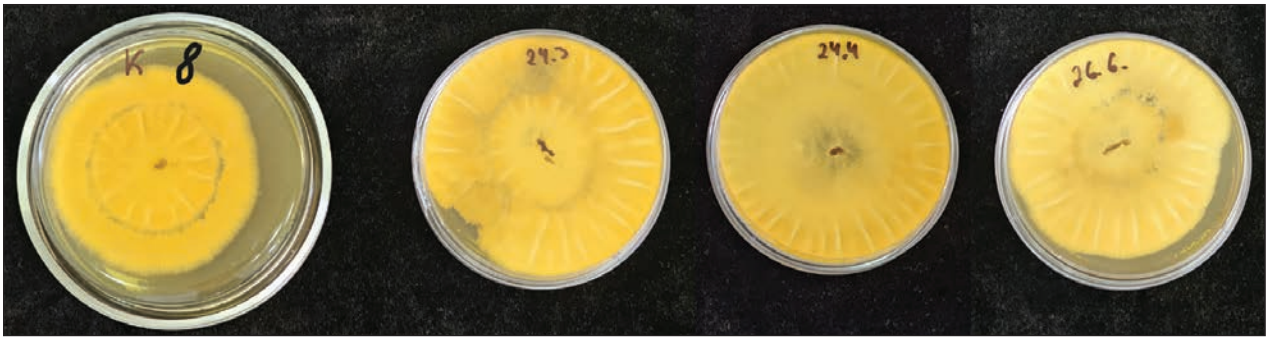

Figure 3. Growth stimulation of *Rhizoctonia solani* 16036 by Antarctic endophytic bacteria. From left to right — control (nutrient media without bacterial metabolites), nutrient media with metabolites synthesized by *Pseudomonas sp.* 24.3, *Pseudomonas yamanorum* 24.4, and *Lysinibacillus macrolides* 26.6, respectively

and the associated microbial communities. These challenging conditions likely select for endophytic bacteria with specialized metabolic adaptations, including the production of antifungal compounds. Bacterial cultures were maintained at 4 °C and 15 °C to simulate relevant environmental conditions. However, the observed temperature tolerance of studied strains (Table) suggests their potential for broader ecological interactions, possibly involving other hosts such as mammals or birds.

Some strains (10.4, 24.3, 25.2, 26.2, 26.4, 26.7, 39.4, and 39.12) were capable of psychrophilic growth. However, most strains showed active biomass growth at 37 °C and 42 °C, which could be evidence of mammals and/or birds as intermediate hosts.

Besides, strains *Arthrobacter psychrochitiniphilus* 15.6, *Pseudomonas yamanorum* 24.4, *Hafnia psychrotolerans* 25.2, and two *Pseudomonas sp.* isolates (24.3 and 39.4) showed antifungal potential against diverse species of phytopathogenic fungi (Fig. 1).

Interestingly, only one of the two tested strains of *Arthrobacter psychrochitiniphilus* had antifungal activity. This isolate was effective against almost all phytopathogenic fungi used in this study.

3.2 Profungal activity

While endophytic bacteria are often considered beneficial to plant health, they may inadvertently stimulate the growth of plant pathogenic fungi under specific conditions. This seemingly paradoxical effect can be attributed to several factors, including nutrient release and indirect interactions. Besides the antifungal activity of the studied bacteria, growth-promotion (the effect of bacterial metabolites on fungal biomass) was noticed as well (Figs. 2, 3).

Despite the fungal growth-promotion effect, these three endophytic bacterial strains also showed plant growth-promoting (PGP) activities. *Lysinibacillus macrolides* 26.6 and *Pseudomonas sp.* 24.3 were positive in BSF and ammonia production. BSF are surface-active compounds produced by microorganisms that enhance microbial adaptation in the rhizosphere by promoting biofilm formation on plant roots and improving bacterial motility. Importantly, these compounds are considered effective antifungal agents against pathogenic fungi, including *Fusarium* spp. and *Aspergillus* spp. (Styczynski et al., 2022). Besides, *Lysinibacillus macrolides* 26.6 was also able to synthesize IAA. *Lysinibacillus spp.* are well-known for entomopathogenic activity and were reported to have a biocontrol potential against a significant spectrum of phytopathogens, including but not limited to such species as *Rhizoctonia solani, Botrytis cinerea, Fusarium oxysporum*, and *Alternaria alternata* (Ahsan & Shimizu, 2021). However, antifungal activity in different studies within a group of bacteria was species- and strain-dependent due to the possible presence and expression of a wide range of genes responsible for PGP and biocontrol activities in every particular case. Therefore, there is a need for further investigation of the anti- and pro-fungal activity of particular endophytic bacteria.

4 Discussion

The fungi used in the study (*Nigrospora oryzae*, *Botrytis cinerea*, *Fusarium oxysporum*, *Alternaria alternata, Sclerotinia sclerotiorum*, and *Rhizoctonia solani*) are widespread plant pathogens that cause significant economic losses in agriculture. They can infect a broad range of crops, including agricultural and ornamental plants (Soltani Nejad et al., 2017; Lotfalinezhad et al., 2024). *Sclerotinia sclerotiourum* and *R. solani* are well-established models in phytopatology research. Their pathogenicity is linked to the production of toxins and enzymes that degrade plant tissues (Aggeli et al., 2020; Wu et al., 2023). Understanding the influence of environmental factors on the growth and development of these pathogens is crucial for developing effective disease management strategies.

A microorganism with plant growth-promoting potential could reprogram the growth of its associated plant host. This effect could be achieved through physiological signaling pathways, specifically during fungal pathogenic attacks, which leads to changes in the phytohormonal profile of the plant (Shahzad et al., 2017).

Endophytes can inhibit pathogen invasion directly and indirectly. Direct antagonism involves competition, where endophytes restrict pathogen growth, often by producing inhibitory metabolites. Indirectly, endophytes can enhance plant defenses by stimulating the host's immune system and upregulating the defense-related genes (Muñoz Torres et al., 2021). Endophytic bacteria produce antimicrobial compounds, induce host plant defense responses, and compete for resources (Zhang et al., 2022). A key mechanism involves the biosynthesis of secondary metabolites, such as phenolics, alkaloids, and terpenoids, which can damage fungal cells by membrane disruption, enzymatic pathways interference, and inhibition of the synthesis of essential cellular components (Chadha et al., 2014; Deshmukh et al., 2018; Midhun & Jyothis, 2021). Moreover, endophytic bacteria can stimulate plant defense responses, activating signaling pathways and inducing the production of defense-related compounds. By colonizing plant tissues, these beneficial bacteria compete with pathogens not just for essential nutrients but for space as well, thereby limiting the fungal potential to cause infections (Fadiji & Babalola, 2020). Ongoing research continues to unveil the diverse mechanisms used by endophytic bacteria in their bacterial-fungal interactions, highlighting their potential as a valuable source of promising antifungal agents and sustainable biopesticides for further use in agriculture and human health studies (Deshmukh et al., 2018; Midhun & Jyothis, 2021).

Among bacteria, most endophytic strains of the *Bacillus* and *Pseudomonas* genera showed antifungal potential towards agriculturally important plant pathogens. They have been reported to suppress fungal pathogen growth by secreting antibiotics and siderophores and inducing the plant's systemic resistance (Meliah et al., 2021). In PGP and biocontrol activities, biofilm-forming and quorum-sensing strategies could successfully protect plants against fungi. For example, Bauer et al. (2016) described *Pseudomonas chlororaphis* subsp. *aurantiaca* PB-St2 as an endophytic biocontrol strain in which the acyl-homoserine lactone signaling system might accommodate a dual role of related genes *phzI, csaI*, and *aurI* in biocontrol activity as well. Similarly, another endophytic pseudomonad, *Pseudomonas putida*, modified with an antifungal *phz* gene, reduced the fungal population on soils in a wheat field (Glandorf et al., 2001). *Pseudomonas* species are famous for producing a wide range of antifungal metabolites like 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, or hydrogen cyanide, which suppress the growth of different species of phytopathogenic fungi (Sokołowski et al., 2024). Recently, another bacteria

Burkholderia gladioli MB39, originally obtained from samples in Antarctica, has shown antifungal potential against *Penicillium digitatum* and *Macrophomina phaseolina*, by affecting mycelial growth and cell morphology (Meng et al., 2023).

Within a plant-microbial interaction system, both partners affect each other's metabolic properties and cause changes in the physiological profile, even in the symbiotic way of interactions. This can promote the plant's or the fungus's growth under normal and extreme conditions (Muñoz Torres et al., 2021). Alves et al. (2019) revealed such fungal genera as *Cladophialophora*, *Cladosporium*, and *Penicillium* in a wide range of Antarctic terrestrial econiches. Different types of rocks may shelter a diverse fungal consortium, which are developed various interactions with different species, suggesting a complex microbial web that could also include endophytic bacteria. Recent studies of biodiversity and ecological interactions of microbial communities in the Antarctic region have shown intriguing insights for the plant-bacteria-fungi triangle. For instance, Znój et al. (2022) characterized a diverse assemblage of bacteria associated with the roots of *Deschampsia antarctica*, including the plant pathogen *Clavibacter michiganensis* alongside a significant presence of Polyangiaceae family members. Notably, many members of the Polyangiaceae are known for their predatory lifestyle and the production of antimicrobial secondary metabolites. Similarly, Piłsyk et al. (2024) demonstrated that Antarctic grasses harbor a diverse fungal community, with a notable prevalence of strains exhibiting plant-beneficial traits, such as strong chitinolytic activity. Chitinolytic enzymes play are crucial in controlling a wide range of pests and pathogens, including phytopathogenic fungi, nematodes, and insects. Interestingly, metabarcoding analysis of antarctic mosses affected by the so-called "fairy ring" disease (Rosa et al., 2021) confirmed the abundant presence of various phytopathogenic fungi species. A significant increase in fungal community diversity, richness, and dominance was observed from healthy to infected to dead moss samples. However, the opposite tendency was observed with endophytic bacteria associated with these mosses. Endophytic-pathogenic interactions in the region could be altered due to changing environmental conditions such as temperature, water availability, wind, nutrient content, host presence and its kind, etc.

5 Conclusions

In conclusion, the endophytes exhibited significant antifungal activity, suggesting their potential as biocontrol agents to combat plant diseases. Our findings demonstrate the crucial role of endophytic bacteria in promoting plant growth and stress tolerance. These microorganisms can produce a diverse range of secondary metabolites that could enhance plant defense mechanisms and improve nutrient acquisition not just for the plant but for its symbiotic fungi as well. Further investigation into the mechanisms of action of these endophytes and the optimization of their application in agricultural settings could lead to the development of sustainable and environmentally friendly disease management strategies.

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Conflict of Interest. The authors declare no conflict of interest.

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Потенціал антарктичних ендофітних бактерій як агентів біоконтролю

Реферат. Антарктичні ендофітні бактерії, що асоційовані з судинними рослинами та адаптовані до суворих умов навколишнього середовища, мають унікальні метаболічні властивості, що можуть впливати на взаємодію рослин і мікроорганізмів. У цьому дослідженні ми вивчили вплив 15 бактеріальних штамів, що стимулюють ріст рослин, ізольованих з єдиних аборигенних видів судинних рослин Антарктичного регіону — *Deschampsia antarctica* і *Colobanthus quitensis* — на ріст фітопатогенних грибів. Також було показано, що досліджувані бактерії здатні синтезувати біосурфактанти, аміак та гормоноподібні сполуки класу ауксинів, які визначають як ріст-стимулювальні сполуки для рослин. Крім того, досліджувані штами демонструють значний приріст біомаси за широкого діапазону температур, що може свідчити про їх взаємодії не лише з рослинами-господарями, а й з вищими теплокровними організмами. Хоча зазначені ендофітні бактерії виявили значну антифунгальну активність проти штамів мікроміцетів, що мають сільськогосподарське значення, ми також спостерігали стимуляцію росту грибів певними штамами з досліджуваних бактерій, тобто профунгальну активність. Така подвійна роль ендофітів підкреслює складний і контекстозалежний характер взаємодії рослин і мікроорганізмів, особливо в умовах Антарктичного регіону. Наші результати свідчать про те, що вплив ендофітів на макроорганізм рослини може бути багатогранним. Окрім того, що бактерії-ендофіти можуть безпосередньо пригнічувати патогенні мікроміцети, вони також можуть опосередковано впливати на мікробіом рослин, що потенційно призводить як до позитивних, так і негативних наслідків. Подальші дослідження необхідні для з’ясування механізмів, що лежать в основі цих складних взаємодій, та для використання потенціалу антарктичних бактерій-ендофітів для сталого сільського господарства.

Ключові слова: Антарктичний регіон, антифунгальна активність, рослинно-мікробні взаємодії