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ANTIFUNGAL ACTIVITY OF ENDOPHYTIC BACTERIA ASSOCIATED WITH ANTARCTIC VASCULAR PLANTS

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Plants harbor a wide diversity of microorganisms, which play a crucial role in their growth, survival and establishment by conferring enhanced resistance to abiotic stress, allowing plants to grow in extreme conditions. Endophytic bacteria, residing within plant tissues, often exhibit antimicrobial properties. This study investigated the antifungal potential of endophytic bacteria isolated from Antarctic vascular plants. Isolates were screened for their inhibitory activity against a panel of plant pathogenic fungi. Among studied bacteria only 3 strains have shown broad spectrum of antifungal activity – *Arthrobacter psychrochitiniphilus* 15.6, *Pseudomonas yamanorum* 24.4, and *Hafnia* sp. 25.2. The results highlight the potential of Antarctic endophytic bacteria as a promising source of novel bioactive compounds for the development of sustainable biocontrol agents. However, there is a need to identify specific compounds responsible for inhibiting phytopathogens growth. Understanding the mechanisms of antifungal activity is crucial for the effective application of these bacteria in agriculture and other fields.

Keywords: endophytic bacteria, antifungal activity, Antarctic plants

INTRODUCTION

Microorganisms in the Antarctic region have garnered significant attention due to their potential to produce novel bioactive compounds. Extremophilic bacteria and fungi from this region have demonstrated remarkable adaptations to the harsh environmental conditions, which include cold temperatures, high salinity, and intense ultraviolet radiation (Corrêa & Abreu, 2020). In recent years, there has been a growing interest in exploring the diversity and bioprospecting potential of these microorganisms, particularly their antifungal properties (Brunati *et al*., 2009; Godinho *et al*., 2013).

Endophytic bacteria, which are microorganisms that reside within plant tissues without causing any apparent harm to the host, have emerged as a promising source of antifungal compounds (Deshmukh *et al*., 2018). These endophytes have the ability to produce a diverse array of secondary metabolites, including volatile organic compounds, that can inhibit the growth of pathogenic fungi (Deshmukh *et al*., 2018).

The diverse and unique microbial communities found in Antarctica, including those associated with endemic plant and algal species, represent an underexplored source of bioactive compounds (Deshmukh *et al*., 2018). Endophytic bacteria, which reside within the tissues of host organisms without causing any apparent harm, have been identified as a

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particularly promising group for the discovery of novel antifungal agents (Deshmukh *et al*., 2018).

Studies have shown that cold-tolerant fungal genera, such as *Penicillium*, *Aspergillus*, *Beauveria*, and *Cladosporium*, isolated from benthic mats in Antarctic lakes have demonstrated potent antimicrobial activities, including the production of novel antibiotics (Brunati *et al*., 2009). These findings suggest that the endophytic bacterial communities associated with Antarctic plant and algal species may also harbor the potential to produce a wide range of antifungal compounds with diverse structural and functional characteristics.

This research paper aims to investigate the antifungal activity of endophytic bacteria isolated from Antarctic vascular plants.

MATERIALS AND METHODS

Plant Samples Collection

Plant samples were collected during the 25th Ukrainian Antarctic Expedition (January-April 2020) along the Western part of the Antarctic Peninsula (WAP). To isolate endophytic bacteria, surface sterilization of the plants was performed according to Barra *et al*. (2016) with modifications.

Bacterial Species Used in the Study

For this study, 12 bacterial cultures isolated from *D. antarctica* and *C. quitensis* samples were investigated (Iungin *et al*., 2023) and represented in Table 1.

Antifungal Activity Studies

Antifungal activity of bacterial isolates was tested with the agar disk-diffusion method (Elkahoui *et al*., 2012). Six phytopathogenic fungi cultures were obtained from the National collection of D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine (Department of Physiology and Systematics of Micromycetes), *Nigrospora oryzae* 15966, *Fusarium solani* 50718, *Nectria inventa* 3041, *Botrytis cinerea* 16884, *Sclerotinia sclerotirum* 16883, and *Rhizoctonia solani* 16036. A 5-day mycelium of pregrown fungi culture was placed in the middle of the Petri dish with a sterile needle. Overnight bacterial cultures were inoculated equidistantly from the fungus and incubated for five days at 25 °C. The results were presented as a percentage of fungal growth inhibition.

RESULTS AND DISCUSSION

Endophytic Bacteria

The habitat of the vascular plants and the plant communities they create is largely defined by the environmental extreme conditions of the Antarctic continent, which include low temperatures, high levels of UV radiation, and low water availability. These harsh conditions likely select for endophytic bacteria that are adapted to produce a wide range of bioactive secondary metabolites, including those with antifungal properties. Mean temperature in Antarctic is around -10 °C, so bacteria were cultivated at 4 °C and 15 °C to mimic the environmental conditions. However, previous studies have shown the ability of isolated endophytic strains (Table 1) to grow at wide temperature range, suggesting their unique origin and the presence of an intermediate host, such as mammals or birds.

Table 1. Endophytic bacteria associated with Antarctic vascular plants

Species name	Strain number	Host plant	Place of isolation
Siminovitchia terrae	9.1	*D.antarctica*	Lahille Island
Pseudomonas salomonii	10.1	*C. quitensis*	Lahille Island
Psychrobacter arcticus	10.4	*C. quitensis*	Lahille Island
Arthrobacter psychrochitiniphilus	15.6	*D.antarctica*	Ronge Island
Arthrobacter psychrochitiniphilus	16.7	*D.antarctica*	Ronge Island
Agreia sp.	23.2	*D.antarctica*	Santos Peak, Graham Passage
Pseudomonas yamanorum	24.4	*D.antarctica*	Santos Peak, Graham Passage
Hafnia sp.	25.2	*D.antarctica*	Galindez Island, Argentine Islands
Pseudomonas sp.	26.2	*D.antarctica*	Galindez Island, Argentine Islands
Pseudoarthrobacter sp.	26.7	*D.antarctica*	Galindez Island, Argentine Islands
Brachybacterium sp.	39.12	*C. quitensis*	Lagotellerie Island
Kocuria salsicia	40.1	*D.antarctica*	Lagotellerie Island

Half of the studied isolates (10.4, 25.2, 26.2, 26.4, 26.7, 39.12) demonstrated psychrophilic growth ability. However, the majority of isolates showed active biomass growth at 37°C and 42°C. These findings broaden our understanding of plant-microbe interactions in Antarctic vascular plants within the context of the ecosystem's overall functioning.

Antifungal Activity

Endophytic bacteria, which reside within plant tissues without causing any apparent harm to the host, have garnered significant attention in recent years due to their remarkable ability to produce a diverse array of secondary metabolites and enzymes that exhibit potent antifungal properties (Deshmukh *et al*., 2018; Midhun and Mathew, 2021). These endophytic microorganisms have evolved alongside their plant hosts, establishing symbiotic relationships that enable them to thrive in the nutrient-rich environment within the plant tissues (Chadha *et al*., 2014).

Among the isolated bacterial strains, only three – *Arthrobacter psychrochitiniphilus* 15.6, *Pseudomonas yamanorum* 24.4, and *Hafnia* sp. 25.2 – showed antifungal activity against phytopathogenic fungi (Table 2). The other strains had no effect on the growth of the selected fungal strains.

Table 2. Endophytic bacteria antifungal activity

Fungal species name	Inhibition of fungal growth, %		
	Arthrobacter psychrochitiniphilus 15.6	*Pseudomonas yamanorum* 24.4	*Hafnia sp.* 25.2
Nigrospora oryzae 15966	11.13 ± 1.73	–	18.30 ± 1.90
Fusarium solani 50718	33.00 ± 8.00	20.33 ± 1.15	–
Nectria inventa 3041	–	5.10 ± 0.21	21.85 ± 2.03
Botrytis cinerea 16884	18.28 ± 1.58	–	18.90 ± 1.90
Sclerotinia sclerotirum 16883	21.61 ± 2.13	11.43 ± 0.57	–
Rhizoctonia solani 16036	5.96 ± 0.16	3.37 ± 0.2	20.08 ± 0.21

By exploring the diversity and metabolic capabilities of these endophytes, the study seeks to identify potential sources of novel antifungal agents that could be developed for various applications, such as in medicine, agriculture, and industry.

The antifungal activity of endophytic bacteria is attributed to a variety of mechanisms, including the production of antimicrobial compounds, the induction of host plant defense mechanisms, and the competition for resources (Zhang *et al*., 2022). One of the primary mechanisms of antifungal activity in endophytic bacteria is the synthesis of secondary metabolites, such as phenolic compounds, alkaloids, and terpenoids, which can inhibit the growth and development of pathogenic fungi (Deshmukh *et al*., 2018; Midhun and Mathew, 2021). These bioactive molecules can disrupt fungal cell membranes, interfere with enzymatic processes, and hinder the synthesis of essential cellular components, thereby effectively controlling fungal infections (Chadha *et al*., 2014; Zhang *et al*., 2022).

Additionally, endophytic bacteria can stimulate the host plant's innate immune system, triggering the production of defense-related compounds and the activation of signaling pathways that enhance the plant's resistance to fungal pathogens (Fadiji and Babalola, 2020). By colonizing the plant's internal tissues, endophytic bacteria can also outcompete pathogenic fungi for essential nutrients and space, thereby limiting their ability to establish infections (Fadiji and Babalola, 2020).

Ongoing research continues to uncover the diverse mechanisms employed by endophytic bacteria in their antifungal activities, highlighting their potential as a promising source of novel antimicrobial agents and biopesticides for sustainable agriculture and human health applications (Deshmukh *et al*., 2018; Midhun and Mathew, 2021).

CONCLUSIONS

In conclusion, endophytic bacteria possess a remarkable arsenal of mechanisms that contribute to their potent antifungal properties. These include the production of antimicrobial secondary metabolites, the induction of host plant defense responses, and the competitive exclusion of fungal pathogens. The findings of this research could contribute to the development of novel antifungal agents derived from endophytic bacteria isolated from Antarctic plant species.

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