PLANT GROWTH-PROMOTING CHARACTERISTICS OF ANTARCTIC ENDOPHYTIC BACTERIA

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The work is focused on studying bacteria associated with vascular plants in Antarctic region. Climate changes affecting the Antarctic Peninsula favor the successful colonization of ice-free lands by two Antarctic vascular plants (*Deschampsia antarctica* and *Colobanthus quitensis*). Bacteria isolated from *D. antarctica* collected during the 25th Ukrainian Antarctic Expedition (January-April 2020) along the Western part of the Antarctic Peninsula were studied for plant growth-promoting characteristics (nitrogen fixation, phosphate solubilization, cyclic lipopeptide production, exoprotease production, motility and carbohydrate utilization). The heterotrophy of bacterial isolated from *D. antarctica* and the presence of a wide range of saccharolytic enzymes for the utilization of mono- and disaccharides in studied cultures were shown. This may indicate the plasticity of metabolism and the high adaptation potential of microorganisms associated with *D. antarctica*. PGPT of studied bacteria were mostly presented by nitrogen-fixing ability and cyclic lipopeptides synthesis.

Keywords: PGPB, antarctic vascular plants, endophytic bacteria

INTRODUCTION

The arisen interest shown to microorganisms associated with vascular plants of the Antarctic region was not only due to the knowledge about plant-microbial interactions, but also due to the prospects of climate change in the world and the biotechnological potential of these microorganisms. Climate changes affecting the Antarctic Peninsula favor the successful colonization of ice-free lands by two Antarctic vascular plants (*Deschampsia antarctica* and *Colobanthus quitensis*). Bacteria are important for the growth and adaptation of plants to extreme conditions of growth and development.

Antarctic plants have developed adaptation mechanisms that allow them to successfully survive the extreme conditions of the region. Thus, recently it was shown the leading role of micromycetes-endophytes in the tolerance of plants to UV-B and minimizing damage to plant cells (Barrera *et al.*, 2020); stimulation of plant growth by increasing nitrogen mineralization (Oses-Pedraza *et al.*, 2020); improvement of physiological indicators under salt stress due to energy docking and complex formation with Na^+ (Molina-Montenegro *et al.*, 2020). However, there are almost no studies on the development and influence of bacterial endophytes of vascular plants in the Antarctic region.

The understanding of interactions in the "microorganism-plant" system helps to assess the effect of bacteria on the growth and development of plants consisted of its ability to participate in biochemical mechanisms shared with the plant (Compant *et al*., 2021).

The aim of the study was to screen plant growth-promoting traits in endophytic bacteria of antarctic vascular plants.

EXPERIMENTAL

Materials

We have studied 8 bacterial cultures isolated from *D. antarctica* collected during the 25th Ukrainian Antarctic Expedition (January-April 2020) along the Western part of the Antarctic Peninsula.

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Methods

Overnight liquid cultures were obtained on diluted Nutrient Broth medium (HiMedia, Ltd.) in a shaking incubator (26 , 160 rpm).

The ability of bacterial isolates to utilize different carbohydrates was tested with long “Hiss rows” with the use of 9 carbohydrate substrates: glucose, fructose, mannose, galactose, arabinose, xylose, ribose, lactose, sucrose (Gunkova *et al*., 2021).

Nitrogen-fixing activity were checked on Ashby's combined-nitrogen-free medium with sucrose. Bacterial growth was determined by the change of optical density (OD600) and evaluated as + (weak growth), ++ (moderate growth), +++ (abundant growth) (Mohite and Patil, 2022).

Drop collapse assay for cyclic lipopeptides production (CLPs) was performed onto Parafilm. The reduction of the surface tension and the collapse of the droplet (10 µL aliquots of bacterial overnight culture) indicated the presence of surfactants (De Souza *et al.*, 2003).

Motility assay was performed onto 1/5 Nutrient agar (0.3%). 10 µL aliquots of bacterial overnight culture were spot in medium surface. Colony diameter was measured in 24, 48 and 72 h after inoculation on 0.3% 1/5 NA (Ha *et al.*, 2014).

Exoprotease production was tested using skim milk agar (Vazquez *et al.*, 1995). A cleared zone surrounding bacterial growth after incubation for 48 and 72 h at 28°C was the evidence of exoprotease production. Phosphate solubilizing ability was tested on Pikovskaya (PVK) medium (Pikovskaya, 1948) incorporated with $Ca_3(PO_4)_2$.

All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Carbohydrates fermentation ability is both the way to help with bacteria taxonomic identification and to describe possible physiological characteristics of the isolates, predict their range of substrates and enzymes.

Table 1. Saccharolytic enzymes of studied isolates

Isolate	Hexoses					Pentoses		Disaccharides	
№	Glu	Fru	Man	Gal	Ara	Xyl	Ryb	Lac	Suc
10.4	+	-	+	+	-	+	+	-	-
15.6	+	-	-	-	-	+	-	-	-
25.2	+	+	+	+	-	+	+	+	+
26.2	+	-	+	+	-	+	-	-	+-
26.4	+	-	+	+	-	+	-	-	+
26.7	+	+	+	-	-	-	+	+-	+
39.12	+	+	-	-	-	-	+	-	-
40.1	+	+	+	+	+	+	+	+-	+

«+» positive reaction; «+-» doubtful reaction; «-» negative reaction

The most usable substrates were glucose, mannose and xylose. Mannose is an isomer of glucose, a component of many polysaccharides and mixed biopolymers of plant, animal and bacterial origin. Xylose as the main component of polymers in plants is considered one of the most abundant carbohydrates on earth after glucose. Taking into account that the studied isolates are associated with plants, it is quite clear that these carbohydrates could be promising for metabolic use by bacteria. The ability of microorganisms to metabolize a wide range of Carbon and Nitrogen sources, to participate in global cycles of transformation of the main biogenic elements, and to

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function in harsh environments can be the basis of plant adaptation to adverse environmental factors (Singh, 2018).

However, bacteria associated with plants can promote plant growth directly usually by either facilitating resource acquisition (including micronutrients and minerals), or indirectly by decreasing the inhibitory effects of various pathogenic agents on plant growth and development (Glick, 2012). The direct stimulation of plant growth may be a consequence of nitrogen fixation, phosphate solubilization, iron sequestration, synthesis of phytohormones (such as auxins, cytokinins, and gibberellins), or modulation of plant ethylene levels (Gamalero and Glick, 2011) helping plants to overcome stress and support cell metabolism.

That is why the mentioned set of screened plant growth-promoting characteristics was chosen based on the importance of adequate nutrition in low-temperature environment and defence system against numerous of pathogens (Pršić and Ongena, 2020). All studied isolates have shown plant growth-promoting traits (Table 2).

Table 2. PGPT shown by antarctic bacterial isolates

Isolate №	CLPs	N_2-fixing activity**	Motility
10.4	+	+++	-
15.6	-	+	+
24.4	+	+++	+
25.2	+	+++	+
26.2	+	+++	+
26.7	+	++	-
39.12	+	-	+
40.1	+	-	-

* Phosphate solubilizing ability and exoprotease production are not shown because it was not detected;

** + weak growth, ++ moderate growth, +++ abundant growth in Ashby's medium.

The most abundant were nitrogen-fixing activity, cyclic lipopeptides production and motility. These traits play important role in plant colonization and promoting the growth of plants in harsh environments. Nitrogen is known as one of the limiting factors regarding plant growth (Puri *et al*., 2018). Biological nitrogen fixation plays a great role in subsidizing plants with nitrogen in such limiting or low-mobility environments as Antarctic region. Phosphorus is one of the six elements essential for plant growth. The majority of phosphate solubilizing bacteria affiliates with Paenibacillus, Bacillus, Pseudomonas, Lactococcus, Enterobacter and Alcaligenes (Li *et al*., 2021). Although there were Bacillus and Pseudomonas among studied isolates there was no evidences of phosphate solubilizing ability as well as exoproteases synthesis.

The evidences of CLPs were shown by almost all isolates. Despite the fact that CLPs are known as biocontrol molecules, their role is believed more complicated than this (Wan *et al*., 2021). CLPs exhibit interesting biological activities including interactions with biofilms (Balleza *et al*., 2019) which affect not pathogens only but could manage colonization activity and the balance among endophytic community itself.

CONCLUSIONS

The heterotrophy of bacterial isolated from *Deschampsia antarctica* and the presence of a wide range of saccharolytic enzymes for the utilization of mono- and disaccharides in these cultures were shown. This may indicate the plasticity of metabolism and the high adaptation potential of microorganisms associated with *D. antarctica.* PGPT of studied bacteria were mostly presented by nitrogen-fixing ability and cyclic lipopeptides synthesis.

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