

S. Tistechok<sup>1</sup>, <https://orcid.org/0000-0003-2116-746X>, L-1683-2017,

M. Skvortsova<sup>1</sup>,

A. Luzhetskyy<sup>3</sup>, <https://www.scopus.com/authid/detail.uri?origin=resultslist&authorId=57204143369&zone=>

V. Fedorenko<sup>1</sup>, <https://orcid.org/0000-0002-7672-1897>, K-1792-2014,

I. Parnikoza<sup>2,4</sup>, <https://orcid.org/0000-0002-0490-8134>, O-2781-2019,

O. Gromyko<sup>1,2,\*</sup>, <https://orcid.org/0000-0002-8107-0128>, K-9562-2017

<sup>1</sup> Ivan Franko National University of Lviv, 4 Hrushevskogo Str., Lviv, 79005, Ukraine

<sup>2</sup> State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine, 16 Taras Shevchenko Blvd., Kyiv, 01601, Ukraine

<sup>3</sup> Saarland University, Saarbrücken, UdS Campus C2.3, Saarbrücken, 66123, Germany

<sup>4</sup> Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Akad. Zabolotnogo Str., Kyiv, 03143, Ukraine

\* Corresponding author: [oleksandr.gromyko@lnu.edu.ua](mailto:oleksandr.gromyko@lnu.edu.ua)

## ANTAGONISTIC AND PLANT GROWTH PROMOTING PROPERTIES OF ACTINOMYCETES FROM RHIZOSPHERE *DESCHAMPSIA ANTARCTICA* É. DESV. (GALINDEZ ISLAND, ANTARCTICA)

**ABSTRACT.** **Aim.** The isolation of actinomycetes from *Deschampsia antarctica* É. Desv. (Galindez Island, Antarctica) rhizosphere and determine their ability to produce compounds with antimicrobial and plant growth promotional properties. **Methods.** Actinomycetes were isolated using the three different methods: direct inoculation, the roots treatment with an aqueous solution of phenol and heated at 100 °C for 60 minutes. To study the antibacterial activity of actinomycetes, they were plated on the Oatmeal medium and flooded by 0,7% agar with specific test-culture. Antifungal activities were studied by putting the agar block with fungal culture on Petri plates with cultures of actinomycetes. An activity index was determined by the ratio of the diameter zone of the inhibit growth test-cultures to the diameter of the actinomycetes colonies. Plant growth promotion properties were studied by commonly accepted methods. **Results.** 35 psychotolerant isolates were identified among the 43 actinomycetes isolated from *D. antarctica* rhizosphere. Almost 42% of actinomycetes isolates were antagonists at least one of typical strain of phytopathogenic bacteria (*Pseudomonas savastanoi* pv. *phaseolicola*, *Xanthomonas campestris* pv. *campestris*, *Agrobacterium tumefaciens*, *Erwinia amylovora*) or fungi (*Fusarium oxysporum*, *Botrytis cinerea*, *Alternaria alternata*). An activity index of most isolates was 3–6, in some isolates — 30–32. The potential plant growth promotion properties of isolates were evaluated. 11 isolates of actinomycetes produced indolyl-3-acetic acid (the level of synthesis was 21,0–62,5 µg/ml), 27 isolates produced siderophores and 6 isolates solubilized phosphorus compounds. **Conclusions.** Antimicrobial and plant growth promotional properties of the actinomycetes from rhizosphere *D. antarctica* were evaluated. Phytopathogenic bacteria and fungi antagonists were identified. A number of isolates were characterized by plant growth promotional properties that are combined with the synthesis of antimicrobial compounds. Such properties of isolated actinomycetes may play an important role in the adaptation of Antarctic plants to extreme conditions of existence. The described actinomycetes can be a source of new biologically active compounds and genes that control their biosynthesis.

**Keywords:** Antarctic actinomycetes, rhizospheric microorganisms, antimicrobial activity, plant growth promotional properties.

### INTRODUCTION

Actinomycetes are gram-positive bacteria of the class *Actinobacteria*, which are characterized by a high GC

Cite: Tistechok S., Skvortsova M., Luzhetskyy A., Fedorenko V., Parnikoza I., Gromyko O. Antagonistic and Plant Growth Promoting Properties of Actinomycetes from Rhizosphere *Deschampsia antarctica* É. Desv. (Galindez Island, Antarctica). *Ukrainian Antarctic Journal*, 2019. № 1(18), 169–177.

content in DNA. Some genera of actinomycetes (*Streptomyces*, *Streptoverticillium* et al.) form branched mycelium (Petrus et al, 2014). These bacteria are a major component of soil microbial populations and common in fresh and salt water bodies.

Beginning in the mid-twentieth century actinomycetes (mainly the *Streptomyces* genus) are the main objects of microbial biotechnology as producers most of the antibiotics known today. Antimicro-

bial, antitumor, antiparasitic and other preparations based on natural products from actinomycetes are widely used in medicine and veterinary (Cragg & Newman, 2013; Baltz, 2019). Actinomycetes are also a source of a wide range of bioactive molecules and play important ecological roles as soil-forming bacteria, destructors of complex polymers, plant and animal symbionts (Lewin et al, 2016; Seipke et al, 2012). These bacteria are an integral part of the rhizosphere. Some actinomycetes species colonize the root system as endophytes which can live into the intercellular space of the plants (Dinesh et al, 2017). In the “plant-actinomycetes” system has usually the mutualistic type of symbiosis. The plant’s root exudate includes carbon, nitrogen and other elements for nutrition of the actinomycetes. In turn, actinomycetes produce antibiotics and enzymes that determine their ability to inhibit growth of phytopathogenic bacteria, fungi, nematodes etc. In addition, actinomycetes are capable to phytohormone synthesis, siderophore synthesis and nitrogen fixation. They can also solubilize insoluble elements of plant mineral nutrition, which stimulates their growth (Bhatti et al, 2017).

Today, microorganisms in particular actinomycetes, which inhabit in the poorly studied natural biotopes, are of great biosynthetic interest. Antarctica is one of the most isolated places of the Earth. Periods of low air temperatures, high levels of UV radiation, nutrient deficiency led to the formation of groups of microorganisms with unique properties. In the many studies were characterized the phylogenetic diversity of bacteria that inhabit the Antarctic Islands (King George, Sainte, Deception, Livingston, Crescent, etc.), which belong to such major groups as *Bacterioides*, *Firmicutes*, *Actinobacteria*, *Proteobacteria* (Wynn-Williams, 1996; Gonzalez-Rocha. et al., 2017; Molina-Montenegro et al, 2019). Antarctic actinomycetes from soils and marine sediments showed a broad spectrum of antimicrobial activity and great biosynthetic potential as a source of antibiotics, enzymes and other bioactive compounds (Chean et al, 2015; Núñez-Montero et al., 2018; Silva T.R. et al, 2018). Actinomycetes strains of *Arthrobacter* spp. were isolated from the

water of Terra Nova Bay (Ross Sea) and showed activity against gram-positive and gram-negative bacteria (Lo Giudice et al., 2007). Genomic profiling of streptomycetes that were isolated in the Livingston Island territory led to the identification of genes of type II polyketidesynthase, non-ribosomal peptides synthase and biosynthesis gene clusters of polyene and glycopeptide antibiotics (Encheva-Malinova et al., 2014).

There are also many reports about the microbiota of Antarctic vascular plants, in particular *D. antarctica*. In the genomes of *Pseudomonas* sp. and *Janthinobacterium* sp., which inhabit the plant’s phyllosphere identified genes controlling the synthesis of antifreeze proteins (AFPs) (Cid et al, 2017). Streptomycetes were isolated from the rhizosphere of this plant (Livingston Island) and evaluated their antagonistic properties (Encheva et al., 2013). A new species of rhodococcus was isolated from microbial association of *D. antarctica* on the King George Island (Silva L. J et al., 2018).

Relatively small populations of *D. antarctica* are widespread in the Galindez Island (Argentine Islands Archipelago), where is located the Akademik Vernadsky station (Parnikoza et al., 2018). Tashirev et al. (2010) performed a taxonomic evaluation of microbiota of the *D. antarctica* in this island, among which they identified representatives of the Actinomycetales order. However, the biological properties of rhizospheric actinomycetes, the spectrum of synthesized bioactive compounds, and their possible role in the adaptation of vascular plants to the complex environment of the Antarctic are unclear.

The aim of this study was to isolate of actinomycetes from *D. antarctica* rhizosphere of Galindez Island and determine their ability to produce compounds with antimicrobial and plant growth promotional (PGP) properties.

## MATERIALS AND METHODS

Samples of the rhizosphere from *D. antarctica* were collected on Galindez Island, Argentine Islands, maritime Antarctic (65.245875°S, 64.257505°W) during the 21st Ukrainian Antarctic expedition in 2017. Actino-

mycetes were isolated used three different methods: I – roots samples (2 grams) were placed in flasks with 100 ml of sterile tap water and shaken for 15 min; II – roots samples (2 grams) were placed in flasks with 1.5% aqueous phenol solution and shaken for 30 min; III – roots samples (2 grams) were heated for 60 min at a temperature of 100 °C and then how as I method. Methods II and III were applied to inhibit fast-growing microorganisms, including streptomycetes, to isolate slow-growth actinomycetes, such as *Micromonospora* spp. Further, ten-fold serial dilutions of samples were plated ( $10^{-1}$ – $10^{-5}$ ) on the OM (Gromyko, 2012), ISP4 (Gause et al., 1983) and HVA (Zhang, 2011) media. Nalidixic acid (25 µg/ml) and nystatin (50 µg/ml) were added to media that to inhibit the growth other bacteria and fungi. The plates were incubated at 28 °C for 30 days. The actinomycetes colonies were selected with characteristic growth and morphology. Pure cultures of the isolates were stored in TSB medium (Kieser et al., 2000) at –80 °C in 25 % (v/v) of glycerol. These isolates were deposited in the Microbial Culture Collection of Antibiotic Producers Ivan Franko National University of Lviv.

Agar blocks method was used to determine the optimal growing period for actinomycetes. The cultures were inoculated on the OM medium and blocks of Ø 10 mm were cut from it on 7, 10, 14 days of growth. Then they were put on plates with medium LA and test-culture *Bacillus subtilis* ATCC 31324 and incubated at 37 °C for 24h. Detection was performed visually by measuring the growth inhibition zones of the test-culture around blocks with actinomycetes.

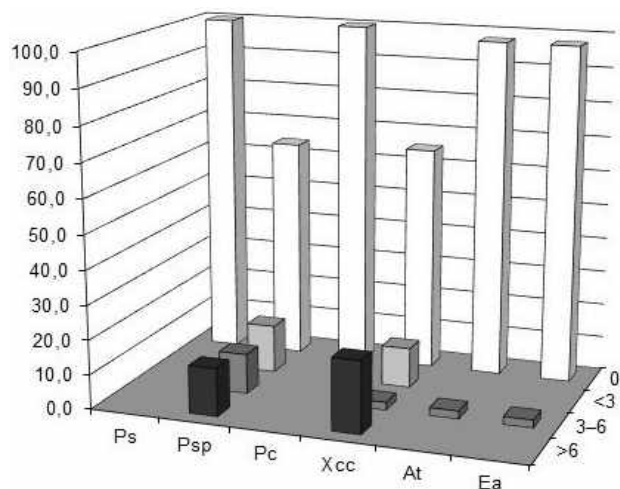
The effect of temperatures on the growth of actinomycetes was determined on the OM medium. The isolates incubated at 4 °C and 28 °C for 14 days. The growth of isolates was evaluated visually and using a ZEISS “Primo Star” light microscope.

Typical strains of phytopathogenic bacteria (*Pseudomonas syringae* IMB 8511, *P. fluorescens* IMB 8573, *P. savastanoi* pv. *phaseolicola* IMB 4012, *Pectobacterium carotovorum* IMB 8982, *Xantomonas campestris* pv. *campestris* IMB8003, *Agrobacterium tumefaciens* IMB 8628 and *Erwinia amylovora* Mi2) and fungi (*Fusarium*

*oxysporum* IMB 54201, *Botrytis cinerea* IMB 2306, *Aspergillus niger* IMB 16706 and *Alternaria alternata* DSM 1102) were used to study the antagonistic properties of isolates. Bacteria and fungi were grown on LA and Sabouraud Dextrose agar (Kieser et al, 2000) respectively. For study antibacterial activity 6 isolates were inoculated on Petri plates with OM medium and incubated at 28 °C for 14 days. Thereafter, plates were flooded by 4 ml of 0,7 % LA containing test-culture ( $10^9$  cells/ml) and incubated for 24 h at 37 °C. In experiments with phytopathogenic fungi, actinomycetes were inoculated and incubated for 5 days as was described above. Further, a block with fungi culture was put in the center of the plates and incubated for 9 days at 23 °C. An activity index (AI) was determined by the ratio of the diameter zone of the inhibit growth test-cultures to the diameter of the actinomycetes colonies. All experiments were performed in triplicate. Statistical data processing was performed using Microsoft Excel determining the average value of IA, error and reliability of IA value by Student’s t-test.

Isolates were inoculated into 10 ml of TSB medium and grown on rotary orbital shaker at 28 °C and 180 rpm for 2 days. The resulting pre-cultures were grown in Erlenmeyer flasks with 30 ml SG medium and 0,2% L-tryptophan (Kieser et al, 2000) for 5 days under the same conditions for determine the ability to produce indolyl-3-acetic acid (IAA). After incubation, 1 ml of culture was centrifuged at 12000 rpm for 2 min, obtained 0,1 ml of supernatant added an equal volume of Salkowski reagent (Sarwar & Kremer, 1995) and incubated at room temperature for 30 min in the dark. The mixture turned pink in the presence of IAA. As a control was used 100 µg of 0,1% IAA with an equal volume Salkowski reagent. The amount of IAA was determined using a pre-constructed IAA calibration curve and ULAB 101 spectrophotometer at  $\lambda = 530$  nm.

Producers of siderophores were determined on the YEM medium. On 7th growth day the colonies were flooded by CAS-indicator solution (Verma et al, 2012) and incubated for 1 h at room temperature. Colonies that synthesized siderophores changed the color of medium from blue to yellow, light yellow or purple.



**Fig. 1.** Antimicrobial activity of rhizosphere actinomycetes of *D. antarctica* of Galindez Island against phytopathogenic bacteria. For axis of abscissas — IA, for axis ordinates — the number of isolates, %. Ps — *P. syringae*, Psp — *P. savastanoi* pv. *phaseolicola*, Pc — *Pec. carotovorum*, Xcc — *X. campestris* pv. *campestris*, At — *A. tumifaciens*, Ea — *E. amylovora*

Ability to solubilize phosphates detected using Muromtsev medium (Muromtsev, 1957). The formation of the enlightenment zones around colonies indicates the ability to solubilize phosphates.

## RESULTS AND DISCUSSION

A total 43 isolates were isolated from *D. antarctica* rhizosphere of Galindez Island. Cultural (growth, shape and color of colonies, the formation of soluble pigments) and morphological (formation of substrate and aerial mycelium) characteristics made it possible to identify them as actinomycetes (Table 1).

**Table 1.** The number of actinomycetes isolated from *D. antarctica* of Galindez Island rhizosphere

| Medium       | Isolation methods  |              |             |
|--------------|--------------------|--------------|-------------|
|              | Direct inoculation | Phenol 1,5 % | 100 °C, 1 h |
| OM           | 8                  | 0            | 12          |
| ISP4         | 7                  | 0            | 1           |
| HVA          | 9                  | 1            | 0           |
| SA           | 5                  | 0            | 0           |
| <b>Total</b> | <b>29</b>          | <b>1</b>     | <b>13</b>   |

As these microorganisms were isolated from the biotope with long periods of low temperatures, their ability to grow under laboratory conditions was investigated at 4 and 28 °C on the OM medium.

Half of the isolates began to form substrate mycelium and 16% formed aerial mycelium on the third day of incubation at 28 °C. On the 6th day, all tested isolates had visible growth, but 42% of them grew rather weakly. On the 14th and 21st day all isolates grew well-accumulated substrate and aerial mycelium. After 3 days incubation at 4 °C of actinomycetes showed no growth. Substrate mycelium appeared in 5% of isolates after 6 days of incubations. At 14 and 21 days 25% of isolates grew well, 36% grew poorly and the rest isolates did not show visible growth. Also we found that continuing incubation of isolates up to 30 days at 4 °C increased the number of isolates growing up to 81%. 19% of isolates did not show signs of growth, although at 28 °C they began to grow by 6–8 days. Thus, 35 isolates from 43 isolated can be related to psychrotolerant actinomycetes, capable to growing both at 4 and 28 °C. Representatives of phylum *Actinobacteria* capable of growing at low temperatures have been identified earlier in the territory of Galindez Island (Romanovskaya et al., 2012). In further experiments, actinomycetes were cultured at 28 °C.

As the investigated actinomycetes showed different growth rates, we selected the optimal duration of cultivation to analyze their antagonistic properties. For this purpose, the gram-positive bacterium *B. subtilis* was used as a test culture. On the 6th, 10th, and 14th days we studied the ability of actinomycetes, which grew well on the 6th day of incubation, to suppress test culture growth. The antibiotic activity of isolates that grew longer was analyzed on the 10th and 14th days of incubation. For this purpose the agar block method was applied. *B. subtilis* growth inhibited 17 of the 43 isolates. Of these, 9 isolates inhibited bacterial growth since 6 days of growth and the inhibition zone did not change at 10 and 14 days of growth. One isolate inhibited the growth of the test culture on the 7 days of growth, and then zone of inhibition disappeared. We assume that the synthesized bioactive compound is unstable. Three isolates began to inhibit the growth of *B. subtilis* on the 10th day and

these inhibition zones unchanged on the 14th day of incubation. Another 5 isolates showed antibiotic activity after 14 days of cultivation. For further experiments to study of antagonistic activity 14-days cultures were used.

To study the antagonistic activity of actinomycetes against phytopathogens 6 species of gram-negative bacteria and 4 species of mycelial fungi were used. Almost 42% of actinomycetes isolates were antagonists at least one of typical strain of phytopathogenic bacteria. 39,4 % of the isolates were *P. savastanoi pv phaseolicola* antagonists (Fig. 1). 13,9 % of isolates had an AI less than 3, in 11,6 % this index ranged from 3 to 6, AI more than 6 had 13,9 % of isolates, in some actinomycetes it reached 30,0. *X. campestris pv. campestris* antagonists were found less (34,8 %). However, there was a majority of actinomycetes with AI bigger than 6 (20,9 %), and the antibiotic activity of individual isolates were AI 32,0. Only 1 isolate showed antimicrobial activity against *A. tumifaciens* and another one against *E. amylovora*, their AI were 3,0 and 4,5 respectively. No *P. syringae* and *Pec. carotovorum* antagonists were detected (Fig. 1).

46,5% of isolates had antifungal activity (Fig. 2). The majority of isolates (39,5%) delayed the growth of *A. alternata*. Most isolates had AI less than 3 and only 4,6% fluctuated between 3–6. It was found less antagonists of *B. cinerea* (32,6%). Among them, there were more isolates with AI 3–6 (7,0%) than AI less than 3. 18,6% were *F. oxysporum* antagonists with an AI less than 3. None of the actinomycetes studied inhibited the *A. niger*.

We evaluated the ability of isolated actinomycetes to produce PGP molecules. The ability rhizosphere microorganisms to produce PGP molecules have important pole in plant growth, especially in the extremal biotopes such as Antarctic region. IAA production of isolates was studied. Also we studied the ability to siderophores production. Siderophores are small peptide molecules that can provide chelating  $Fe^{3+}$  ions in plant-accessible form. The potentially phosphate-mobilizing actinomycetes were screened. We applied a qualitative reaction to IAA using the Salkowski reagent and found 11 producers (25.6%) of this auxin in the *D. antarctica* rhizo-

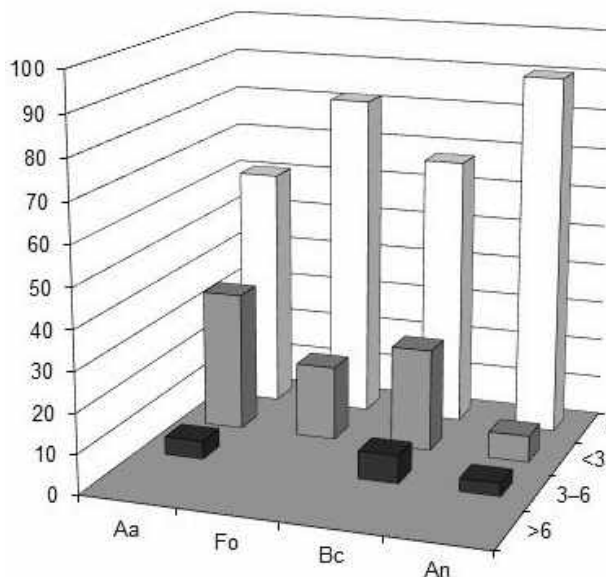


Fig. 2. Antifungal activity of rhizosphere actinomycetes of *D. antarctica* of Galindez Island against phytopathogenic fungi. For axis of abscissas — IA, for axis of ordinates — the number of isolates, %. Aa — *A. alternata*, Fo — *F. oxysporum*, Bc — *B. cinerea*, An — *A. niger*

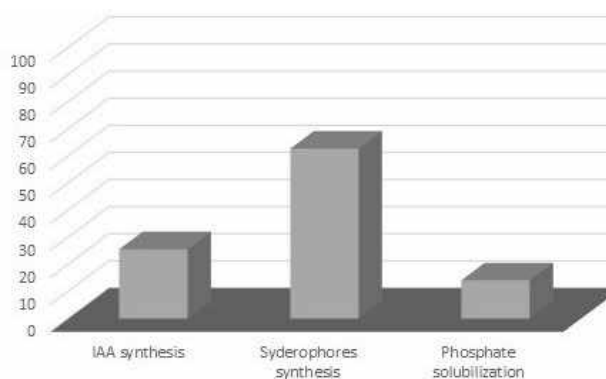


Fig. 3. Ability of rhizosphere actinomycetes isolates of *D. antarctica* of Galindez Island to produce phytostimulant compounds. For axis of ordinates — the number of isolates, %

sphere (Fig. 3). The level of phytohormone synthesis, determined calorimetrically, ranged from 21,0 to 62,5  $\mu\text{g/ml}$ . 62,7% of isolates were able to synthesize siderophores, 14,1 % solubilized phosphates. Two isolates of actinomycetes had all the potential PGP properties.

Table 2 show a variety of antimicrobial and potential PGP properties of isolated actinomycetes. Iso-

Table 2. Properties of some isolates of *D. antarctica* rhizosphere

| Isolat | Activity index           |      |    |      |     |    |                       |     |     |    | PGP properties |   |   |
|--------|--------------------------|------|----|------|-----|----|-----------------------|-----|-----|----|----------------|---|---|
|        | Phytopathogenic bacteria |      |    |      |     |    | Phytopathogenic fungi |     |     |    |                |   |   |
|        | Ps                       | Psp  | Pc | Xcc  | At  | Ea | Aa                    | Fo  | Bc  | An | IAA, µg/ml     | S | P |
| 8      | 0                        | 0    | 0  | 0    | 0   | 0  | 0                     | 0   | 0   | 0  | 62,5           | – | – |
| 1      | 0                        | 0    | 0  | 0    | 0   | 0  | 0                     | 0   | 2,4 | 0  | 0              | + | – |
| 2      | 0                        | 0    | 0  | 0    | 0   | 0  | 0                     | 0   | 3,0 | 0  | 32,5           | – | – |
| 37     | 0                        | 0    | 0  | 0    | 0   | 0  | 0                     | 0   | 1,4 | 0  | 21,5           | + | – |
| 24     | 0                        | 0    | 0  | 0    | 0   | 0  | 1,3                   | 0   | 2,0 | 0  | 0              | + | – |
| 32     | 0                        | 30,0 | 0  | 30,0 | 0   | 0  | 0                     | 0   | 0   | 0  | 0              | + | + |
| 16     | 0                        | 18,5 | 0  | 18,0 | 0   | 0  | 1,6                   | 0   | 4,7 | 0  | 27,5           | + | – |
| 23     | 0                        | 10,7 | 0  | 8,0  | 3,0 | 0  | 1,5                   | 0   | 2,4 | 0  | 26,0           | + | – |
| 27     | 0                        | 5,0  | 0  | 32,0 | 0   | 0  | 1,3                   | 1,5 | 2,8 | 0  | 0              | + | – |
| 28     | 0                        | 15,0 | 0  | 30,0 | 0   | 0  | 1,4                   | 1,2 | 2,4 | 0  | 0              | + | – |
| 33     | 0                        | 11,7 | 0  | 20,0 | 0   | 0  | 1,5                   | 0   | 3,0 | 0  | 22,0           | + | + |

Note: shortening of species names of test cultures as in Figures 1, 2; S — siderophores, P — phosphate solubilization.

lates differed in range and level of antimicrobial activity. Some antagonists, moreover, produced substances that have PGP properties. Isolates 1, 2 and 37 inhibited the growth of *B. cinerea* only. Other actinomycetes were antagonists of several test cultures. Isolate 32 suppressed only bacteria (*P. savastanoi* pv. *phaseolicola* and *X. campestris* pv. *campestris*), and isolate 24 — fungi only (*A. alternata* and *B. cinerea*).

We found isolates with a wide range of antagonistic activity. For example, isolates 27 and 28 inhibited the growth of five test cultures of bacteria and fungi at the same time. Although the activity of most actinomycetes did not exceed AI 6, some isolates (27, 28, 32) showed a high level of antimicrobial action with AI 30,0 – 32,0 against *P. savastanoi* pv. *phaseolicola* and *X. campestris* pv. *campestris*. Along with the antimicrobial action, individual actinomycetes had one or more PGP properties. For example, isolate 32 with a high level of AI against the *P. savastanoi* pv. *phaseolicola* and *X. campestris* pv. *campestris* is able to produce siderophores and solubilize insoluble phosphates. Isolate 33, which is characterized by both antibacterial and antifungal activity, showed all the potential PGP properties that we

have studied. Although isolate 8 did not inhibit any of the test cultures, it had the highest IAA synthesis rate — 62,5 µg/ml.

Recent studies have shown that the *Actinobacteria* class, to which *Actinomycetales* order, is dominant among rhizospheric microorganisms of Antarctic plants, second only to proteobacteria (Molina-Montenegro, 2019). Given this, actinomycetes play a significant role in the life of the plant, including its adaptive capabilities (Hughes et al 2015). The results we obtained indicate that the isolated rhizospheric actinomycetes produce a wide range of bioactive molecules that can be involved in the mechanisms of adaptation of Antarctic plants to low temperatures, lack of nutrition sources and their protection against pathogenic microorganisms. Antarctica is still poorly researched, and therefore there is a high probability to find producers of new bioactive compounds among the actinomycetes of the endemic plants rhizosphere. Further phylogenetic characteristics will give us information about the diversity of actinomycetes of the plant rhizosphere, and with genomic and metabolic profiling we will explore metabolites that isolated actinomycetes synthesize.

## CONCLUSION

43 isolates were isolated from *D. antarctica* rhizosphere of Galindez Island, Antarctic. Among them 35 psychrotolerant actinomycetes were capable of growing both at 4 and 28 °C. We decided to use 14-days cultures because some isolates of actinomycetes showed antibacterial activity after 14 days of cultivation. Antagonists of gram-negative bacteria *P. savastanoi* pv *phaseolicola* IMB 4012, *X. campestris* pv *campestris* IMB8003, *A. tumifaciens* IMB 8628, *E. amylovora* Mi2 and fungi *F. oxysporum* IMB 54201, *B. cinerea* IMB 2306 and *A. alternata* DSM 1102, which cause a wide range of diseases in plants were identified. A number of isolates were characterized by their potential to plant growth stimulation (synthesis of IAA, siderophores and phosphate solubilization). Individual actinomycetes combined these properties. Further study of isolated actinomycetes may reveal their role in the adaptation of *D. antarctica* to Antarctic habitat. Isolated isolates can also be a source of new biologically active substances and genes that control their biosynthesis.

**Acknowledgements:** This study was performed and partially funded under the State Target Scientific and Technical program of research in Antarctica for 2011–2020 within project «Study of Actinomycetes from *Deschampsia antarctica* É. Desv. rhizosphere, Island Galindez (Antarctica)» (contract dated October 1, 2018 № 19).

## REFERENCES

- Baltz, R. H. 2019. Natural product drug discovery in the genomic era: realities, conjectures, misconceptions, and opportunities. *J. Ind Microbiol. Biotechnol.*, 46(3–4), 281–299. doi: 10.1007/s10295-018-2115-4.
- Bhatti, A.A., Haq, S., Bhat, R.A. 2017. Actinomycetes benefaction role in soil and plant health. *Microb. Pathog.*, 111, 458–467. doi: 10.1016/j.micpath.2017.09.036.
- Chean, Y-K., Lee, L-H., Chieng, C-Y.C., Wong, V-L. C. 2015. Isolation, identification and screening of *Actinobacteria* in volcanic soil of Deception Island (the Antarctic) for antimicrobial metabolites. *Polish Polar Research*, 36(1), 67–78. doi: 10.1515/popore-2015-0001.
- Cid, F. P., Inostroza, N. G., Graether, S.P., Bravo, L. A., Jorquera, M. A. 2017. Bacterial community structures and ice recrystallization inhibition activity of bacteria isolated from the phyllosphere of the Antarctic vascular plant *Deschampsia antarctica*. *Polar Biology*, 40(6), 1319–1331. doi: 10.1007/s00300-016-2036-5.
- Dinesh, R., Srinivasan, V., T E S., Anandaraj, M., Srambikkal, H. 2017. Endophytic actinobacteria: Diversity, secondary metabolism and mechanisms to unsilence biosynthetic gene clusters. *Crit Rev. Microbiol.*, 43(5), 546–566. doi: 10.1080/1040841X.2016.1270895.
- Encheva, M., Zaharieva, N., Kenarova, A., Chipev, N., Chipeva, V., Hristova, P., Ivanova, I., Moncheva, P. 2013. Abundance and activity of soil actinomycetes from Livingston Island, Antarctica. *Bulgarian J. of Agricult. Sci.*, 19(2), 68–71.
- Encheva-Malinova, M., Stoyanova, M., Avramova, H., Pavlova, Y., Gocheva, B., Ivanova, I., Moncheva, P. 2014. Antibacterial potential of streptomycete strains from Antarctic soils. *Biotechnology & Biotechnological Equipment*, 28(4), 721–727. doi: 10.1080/13102818.2014.947066.
- Cragg, G. M., Newman, D. J. 2013. Natural products: a continuing source of novel drug leads. *Biochim. Biophys. Acta*, 1830(6), 3670–3695. doi:10.1016/j.bbagen.2013.02.008.
- Gauze, G.F., Preobrazhenskaya, T.P., Sveshnikova, M.A., Terechova, A.P., Maksimova, T.S. 1983. *Key to actinomycetes. Genus Streptomyces*. Moscow: Nauka.
- Gonzales-Rocha, G., Munoz-Cartes, G., Canales-Aguirre, C.B., Lima, C.A., Dominguez-Yevenes, M., Bello-Toledo, H., Hernandez, C.E. 2017. Diversity structure of culturable bacteria isolated from the Fildes peninsula (King George Island, Antarctica): A phylogenetic analysis perspective. *PLoS One*, 12(6), e0179390. doi:10.1371/journal.pone.0179390.
- Gromyko, O. 2012. Antagonistic properties of actinomycetes from the rhizosphere of *Olea europea* L. *Visnyk of the Lviv University. Series Biology*, 59, 209–215.
- Lewin, G. R., Carlos, C., Chevrette, M. G., Horn, H. A., McDonald, B. R., Stankey, R. G., Fox, B. G., Currie, C. R. 2016. Evolution and ecology of *Actinobacteria* and their bioenergy applications. *Annu. Rev. Microbiol.*, 70, 235–254. doi:10.1146/annurev-micro-102215-095748.
- Hughes, K.A., Cowan, D.A., Wilmette, A. 2015. Protection of Antarctic microbial communities — “out of sight, out of mind”. *Front. Microbiol.*, 6, 1–6. doi: 10.3389/fmicb.2015.00151.
- Kieser, T., Bibb, M., Buttner, M., Chater, K., Hopwood, D. 2000. *Practical Streptomyces genetics*, Norwich: John Innes Foundation.
- Lo Giudice, A., Bruni, V., Michaud, L. 2007. Characterization of Antarctic psychrotrophic bacteria with antibacterial activities against terrestrial microorganisms. *J. Basic Microbiol.*, 47, 496–505.
- Molina-Montenegro, M. A., Ballesteros, G. I., Castro-Nallar, E., Meneses, C., Gallardo-Cerda, J., Torres-Díaz, C. 2019. A first insight into the structure and function of rhizosphere microbiota in Antarctic plants using shotgun metagenomic. *Polar Biol.*, 1–11. doi: 10.1007/s00300-019-02556-7.

17. Muromtsev G. S. 1955. On the use of water-insoluble phosphates in soil microbes. *Reports of the All-Union Agricultural Academy*, 5, 35–41.
18. Nunez-Montero, K., Barrientos, L. 2018. Comprehensive narrative review of Bacteria from Antarctic environments as potential sources of novel antibiotic compounds against human pathogens and microorganisms of industrial importance. *Antibiotics (Basel)*, 7(4), doi: 10.3390/antibiotics7040090.
19. Parnikoza, I., Berezkina, A., Moiseyenko, Y., Malanchuk, V., Kunakh, V. 2018. Complex survey of the Argentine Islands and Galindez Island (Maritime Antarctic) as a research area for studying the dynamics of terrestrial vegetation. *Ukrainian Antarctic Journal*, 1(17), 73–101. doi: 10.33275/1727-7485.1(17).2018.34.
20. Petrus, M.L.C., Claessen, D. 2014. Pivotal roles for Streptomyces cell surface polymers in morphological differentiation, attachment and mycelial architecture. *Antonie Van Leeuwenhoek*, 106(1), 127–139. doi: 10.1007/s10482-014-0157-9.
21. Romanovskaya, V.A., Tashyrev, A.B., Gladka, G.V., Tashyeva, A. A. 2012. Temperature Range for Growth of the Antarctic Microorganisms. *Microbiological Journal*, 74(4), 13–19.
22. Sarwar, M., Kremer, R.J. 1995. Determination of bacterially derived auxins by a microplate method. *Lett. Appl. Microbiol.*, 20, 282–85. doi: 10.1111/j.1472-765X.1995.tb00446.x.
23. Seipke, R.F., Kaltenpoth, M., Hutching, M.I. 2012. Streptomyces as symbionts: an emerging and widespread theme? *FEMS Microbiol. Rev.*, 36(4), 862–76. doi: 10.1111/j.1574-6976.2011.00313.x.
24. Silva, T. R., Duarte, A., Passarini, M., Ruiz, A.L.T.G., Franco, C.H., Moraes, C.B., de Melo, I.S., Rodrigues, R.A., Fantinatti-Garboggini, F., Oliveira, V.M. 2018. Bacteria from Antarctic environments: diversity and detection of antimicrobial, antiproliferative, and antiparasitic activities. *Polar Biol.*, 41(7), 1505–1519. doi: 10.1007/s00300-018-2300-y.
25. Silva, L. J., Souza, D. T., Genuario, D. B., Hoyos, H. A. V., Santos, S. N., Rosa, L. H., Zucchi, T. D., Melo, I. S. 2018. *Rhodococcus psychrotolerans* sp. nov., isolated from rhizosphere of *Deschampsia antarctica*. *Antonie Van Leeuwenhoek*, 111(4), 629–636. doi: 10.1007/s10482-017-0983-7.
26. Tashyrev, A.B., Romanovskaya, V.A., Rokitko, P.V., Shilin, S.O., Chernaya, N.A., Tashyeva, A.A. 2010. Microbiological analysis of terrestrial biotopes of the antarctic region. *Microbiological Journal*, 72(2), 3–9.
27. Verma, V, Joshi, K, Mazumdar, B. 2012. Study of siderophore formation in nodule-forming bacterial species. *Res. J. Chem. Sci.*, 2(11), 26–29. doi: 10.1007/s12088-016-0591-7.
28. Wynn-Williams, D.D. 1996. Response of pioneer soil microbial colonists to environmental change in Antarctica. *Microbial Ecology*, 31(2), 177–188. doi: 10.1007/BF00167863.
29. Zhang, J. 2011. Improvement of an isolation medium for actinomycetes. *Modern App. Sci.*, 5(2), 124–27. doi: 10.5539/mas.v5n2p124.

C. Тістечок<sup>1</sup>, М. Скворцова<sup>1</sup>, А. Лужецький<sup>3</sup>, В. Федоренко<sup>1</sup>,  
І. Парнікоза<sup>2, 4</sup>, О. Громико<sup>1, 2, \*</sup>

<sup>1</sup> Львівський національний університет імені Івана Франка,  
вул. Грушевського, 4, м. Львів, 79005, Україна

<sup>2</sup> Державна установа Національний антарктичний науковий центр МОН України  
бульв. Т. Шевченка, 16, м. Київ, 01601, Україна

<sup>3</sup> Університет Саарланду, УдС Будівля С2.3, Саарбрюкен, 66123, ФРН

<sup>4</sup> Інститут молекулярної біології і генетики НАН України,  
вул. Акад. Заболотного, 150, м. Київ, 03143, Україна

\* Автор для кореспонденції: oleksandr.gromyko@lnu.edu.ua

#### АНТАГОНІСТИЧНІ ТА ФІТОСТИМУЛЮВАЛЬНІ ВЛАСТИВОСТІ АКТИНОМІЦЕТІВ РИЗОСФЕРИ *DESCHAMPSIA ANTARCTICA* É. DESV. (О. ГАЛІНДЕЗ, АНТАРКТИКА)

**РЕФЕРАТ. Мета.** Виділення актиноміцетів з ризосфери *Deschampsia antarctica* É. Desv. популяцій антарктичного острова Галіндез, оцінка їхньої здатності пригнічувати фітопатогенні мікроорганізми і продукувати фітостимулювальні сполуки. **Методи.** Актиноміцети виділяли шляхом прямого посіву змивів ризосфери, обробки коренів водним розчином фенолу або прожарювання при 100 °С протягом 60 хв. Антибактерійну активність вивчали шляхом висівання актиноміцетів уколом на вівсяне середовище і заливання 0,7% агаром з певною тест-культурою. Антифунгальні властивості вивчали шляхом викладання агарового блоку з культурою гриба на чашки, засіяні актиноміцетами. За відно-



шенням діаметру зон пригнічення росту тест-культур до діаметру колоній актиноміцетів визначали індекс активності. Фітостимулювальні властивості вивчали загально прийнятими методами. **Результати.** Серед 43 ізолятів актиноміцетів, виділених з ризосфери *D. antarctica*, виявлено 35 психротолерантних ізолятів. Майже 42% ізолятів актиноміцетів були антагоністами хоча б одного типового штаму фітопатогенних бактерій (*Pseudomonas savastanoi* pv. *phaseolicola*, *Xanthomonas campestris* pv. *campestris*, *Agrobacterium tumefaciens*, *Erwinia amylovora*) чи грибів (*Fusarium oxysporum*, *Botrytis cinerea*, *Alternaria alternata*). Індекс активності більшості ізолятів становив 3–6, у деяких з них він сягав 30–32. Оцінено потенційні фітостимулювальні властивості ізолятів. Виявлено 11 продуцентів індоліл-3-оцтової кислоти (рівень синтезу 21,0–62,5 мкг/мл), 27 ізолятів синтезували сидерофори, шість – солюбілізували фосфати. **Висновки.** Оцінено антимікробні та фітостимулювальні властивості актиноміцетів ризосфери *D. antarctica*. Виявлено антагоністів фітопатогенних бактерій і грибів. Для низки ізолятів властиві фітостимулювальні властивості у поєднанні з синтезом антимікробних сполук. Такі властивості виділених актиноміцетів можуть відігравати важливу роль в адаптації антарктичних рослин до екстремальних умов існування. Описані актиноміцети можуть бути джерелом нових біологічно активних речовин та генів, що контролюють їхній біосинтез.

**Ключові слова:** антарктичні актиноміцети, ризосферні мікроорганізми, антимікробна активність, фітостимулювальні властивості.