



## I. Roman, O. Gromyko\*

Ivan Franko National University of Lviv, Lviv, 79005, Ukraine

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

# Plant growth promoting properties of an antarctic strain *Amycolatopsis* sp. Cq 72-27

**Abstract.** Unique biotopes can be a source of new plant growth promotion (PGP) bacteria with rare properties. The Antarctic habitat is an attractive location for research, as it is characterized by many stress factors, and the local microbiota is under permanent selective pressure. We believe that the rhizosphere bacteria of this habitat may have important PGP properties that can be used in agriculture. A variety of research methods were used in this work: the molecular genetic technique to establish the gene sequence, chemical to test the ability to produce nitrite, ammonia, and indole acetic acid, microbiological to investigate the cultured properties of the strain, as well as antagonistic and PGP activities. We found that the strain belongs to the genus *Amycolatopsis*. It showed antagonistic activity against phytopathogenic bacteria (*Xanthomonas campestris* pv. *campestris* IMB8003 and *Bacillus subtilis* ATCC 31324) and fungi (*Alternaria alternata* DSM 1102, *Fusarium oxysporum* IMB 54201, *Aspergillus niger* IMB 16706), and also demonstrated some PGP properties (solubilization of phosphorus and zinc and production of nitrite and ammonia). Inoculation of wheat seeds with spores of this strain promoted germination and growth of seedlings. The strain has demonstrated properties that make it a promising basis for developing biofertilizers that can be used in agriculture.

**Keywords:** antarctic actinomycetes, biocontrol, plant growth-promoting bacteria, rhizospheric bacteria

## 1 Introduction

Microorganisms play an important role in plant nutrition and development, forming close relationships in the root zone of plants. In natural ecosystems, microorganisms are involved in improving the mineral nutrition of plants, synthesizing phytohormones, and inhibiting the development of phytopathogenic bacteria and fungi (Cueva-Yesquén et al., 2021). At the same time, agroecosystems have a poor rhizosphere microbiome due to rare crop rotation and negative biotic and abiotic factors (French et al., 2021). Recent studies show that some rhizosphere bacteria, for example, methan-

otrophs, *Bacteroidetes*, and *Stenotrophomonas*, were found predominantly in wild plants of rice, common bean, or wheat, but not in the domestic forms (Martínez-Romero et al., 2020). Bacteria-based biological products are increasingly used to optimize or improve microbial diversity and to reduce the negative effects of salinity, heavy metals, and phytopathogenic organisms (Majeed et al., 2018; Kumar et al., 2020). Thus, plant growth promotion bacteria (PGPB) can be an effective alternative to traditional fertilizers and pesticides to increase crop productivity in an environmentally stable and sustainable way. Actinomycetes can be promising organisms for new biological products.

Actinomycetes are a group of prokaryotes that can form branched mycelium and reproduce mainly by spores. An important characteristic of actinomycetes is their ability to synthesize complex secondary metabolites and enzymes (Gohain et al., 2020). Because of these properties, actinomycetes, particularly *Streptomyces*, are producers of many antibiotics widely used in agriculture and medicine (Hutchings et al., 2019). They also play an important role as biocontrol agents in the environment, particularly in the rhizosphere of plants. Plant growth promotion actinomycetes provide mineral nutrition for plants and produce phytohormones, antibiotics, and fungicides (Sathya et al., 2017). In addition, actinomycetes can synthesize phytohormone-like compounds, such as pteridic acid, which stimulate plant growth (Igarashi, 2004). In contrast to other PGPBs, actinomycetes easily tolerate adverse conditions as spores and often enter the plant's endosphere, forming strong plant-bacterial relationships (Singh & Dubey, 2018).

Extreme environments are promising sources of microorganisms that have developed unique survival strategies under selective pressure and are likely to produce unusual compounds. Antarctica remains one of the most extreme habitats, characterized by low temperatures, high UV levels, strong winds, absence of bioavailable water, and low nutrient content (Núñez-Montero & Barrientos, 2018). The presence of plants in this region indicates successful adaptation to adverse conditions, but the role of microorganisms in this process cannot be denied. Rihan and colleagues (2017) showed that bacterial cold shock proteins increase plants' resistance to cold stress. In addition, Antarctic rhizosphere bacteria can improve the mineral nutrition of plants by fixing atmospheric nitrogen, solubilizing phosphorus, decomposing complex biomolecules, and producing chemical compounds that can chelate metals and change the surface tension of water (Styczynski et al., 2022).

Although there are data on the PGP properties of Antarctic actinomycetes (Tistechok et al., 2019), the interaction of these bacteria with plants and the impact on plant germination and growth remains to be explored.

## 2 Materials and methods

### 2.1 Bacterial and fungal strains, plants

Isolate Cq 72-27 was obtained from the Culture Collection of Microorganisms – producers of antibiotics of Ivan Franko Lviv National University (<http://lv-microbcollect.lviv.ua/en/>), where it had been isolated from the rhizosphere of the antarctic pearlwort (*Colobanthus quitensis* (Kunth) Bartl.), collected at Port Charcot, Booth Island, Antarctic Peninsula. The phytopathogens used in the study included bacteria *Pseudomonas syringae* IMB 8511, *Xanthomonas campestris* pv. *campestris* IMB8003, *Agrobacterium tumefaciens* IMB 8628, and *Bacillus subtilis* ATCC 31324 and fungi *Alternaria alternata* DSM 1102, *Fusarium oxysporum* IMB 54201, *Aspergillus niger* IMB 16706. All bacteria and fungi were obtained from the same collection. For the plant experiments, wheat seeds of the Opal cultivar (Zakhidnyy Buh, Ukraine) were used.

### 2.2 Bacterial identification

The biomass of the isolate Cq 72-27 was collected from a Petri dish and incubated for 30 min in lysozyme solution (5 mg/ml) at 37 °C. The obtained protoplasts were used to isolate bacterial DNA by the salting out method (Kieser et al., 2000). Primers 5F (AGAGTTT-GATYMTGGCTCAG), 1510R (TACGGYTACCT-TGTTACGACTT), rpoBPF (GAGCGCATGACC-ACCCAGGACCTCGAGGC), and rpoBPR (CCT-CGTAGTT GTGACCCTCCCACGGCATGA) were used for the amplification of 16s rRNA and rpoB genes, respectively. PCR was performed using the following program: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and then extension at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. The obtained PCR products were purified using the spin column method (QIAGEN, USA) and sequenced by the Sanger method (Explogen, Ukraine). The obtained data were analyzed in the Geneious Prime software, homologs were searched in the National Center for Biotechnology Information (NCBI) database, and alignment was performed by the T-Coffee algorithm (Notredame et al., 2000). The phylogenetic tree was constructed in the Mega 11 soft-

ware (Tamura et al., 2021) based on the neighbor-joining principle with a bootstrap test (1000 replicates). 16S rRNA gene and the *rpoB* gene were used for multilocus sequence analysis (MLSA) (Glaeser & Kämpfer, 2015).

### 2.3 Cultural properties

Cultural characteristics of the strain were observed after 14 days of incubation on Yeast Malt Agar (International Streptomyces project medium 2 (ISP 2)), Oatmeal Agar (ISP 3), Inorganic Salts-Starch Agar (ISP 4), Glycerol-Asparagine Agar (ISP 5), Peptone Yeast Extract Iron Agar (ISP 6), and Tyrosine Agar (ISP 7) (Shirling & Gottlieb, 1966). The growth at different temperatures (4, 15, 28, 37, and 45 °C), NaCl concentrations (0, 2.5, 5, 7.5, and 10 % w/v), Na<sub>2</sub>SO<sub>4</sub> concentrations (0, 2.5, 5 % w/v), and pH range (pH 4.0–10.0, at intervals of 1.0 pH unit) was tested on ISP 2 agar.

### 2.4 PGP activities

The production of indole acetic acid (IAA) was performed in liquid ISP 2 medium, supplemented with 2 mg/ml tryptophan, at 28 °C with rotary shaking set to 180 rpm, in the dark. After 7 days of incubation, the cultures were centrifuged (10.000 rpm for 5 min), and supernatants were used for IAA quantification. Salkowski reagent (1.2 g of FeCl<sub>3</sub> dissolved in 100 ml 35% perchloric acid) was added to the supernatant at a 2:1 ratio, and the optical density (OD) was measured at 530 nm. As controls, IAA (Sfera Sim, Ukraine) and uninoculated media were used (Tirry et al., 2018).

The phosphate solubilization activity was estimated using the Pikovskaya medium (Shekhar Nautiyal, 1999). The isolate was incubated at 28 °C for 7 days. A halo zone around the areas of bacterial growth indicated the inorganic phosphate solubilization potential (Tirry et al., 2018).

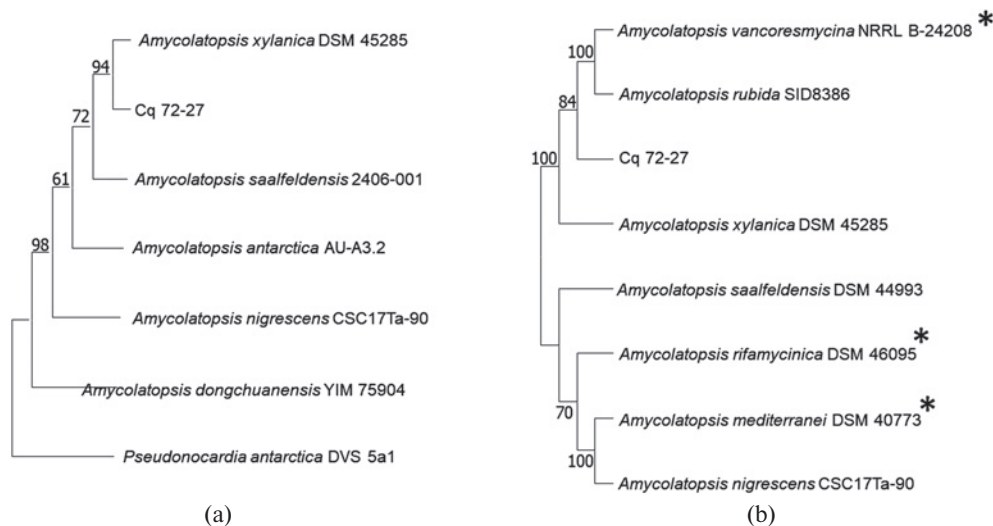
The zinc solubilization activity was estimated using the Minimal Salts medium supplemented with 0.1% ZnO. The isolate was inoculated on plates and incubated at 28 °C for 7 days. A halo zone around the areas of bacterial growth indicated the zinc solubilization potential (Bhakat et al., 2021).

Siderophores were produced on the Yeast Extract Mannitol medium (Yamal et al., 2013). After 7 days of incubation at 28 °C, the culture was covered with 2 ml of the chrome azurol S solution (Louden et al., 2011). The change of color around the areas of bacterial growth indicated the production of siderophores.

The nitrate and nitrite reduction activity assays were performed in liquid MSM medium, with some modifications (ammonium sulfate was replaced by 1 g/l potassium nitrate), at 28 °C with rotary shaking set to 180 rpm. After 7 days of incubation, cultures were centrifuged (6.000 rpm for 5 min), and supernatants were used to detect nitrite and ammonia. To detect the nitrite, 0.25 g of the nitrate test mixture (10.57 g of citric acid and 0.857 g of Griess reagent) was poured into a test tube, and 5 ml of supernatant was added. The tube was shaken for 15 min, and the appearance of pink color indicated nitrite. As controls, sodium nitrite (Sfera Sim, Ukraine) and uninoculated media were used (Singh, 1988). To detect ammonia, we added 10 ml supernatant and 0.5 ml 50% potassium sodium tartrate solution and mixed the contents of the tube. Then we added 0.5 ml Nessler's reagent (Sfera Sim, Ukraine) and left the mixture for 15 min. The appearance of yellow color indicated ammonia. For controls, ammonium sulfate (Sfera Sim, Ukraine) and uninoculated media were used (Zhao et al., 2019).

### 2.5 Detection of antimicrobial and antifungal activities

The antimicrobial and antifungal activities were detected with the DNPM (40 g dextrin, 7.5 g soytone, 5 g dry yeast, 21 g 3-(N-morpholino)propanesulfonic acid (MOPS), 15 g agar, 1000 ml distilled H<sub>2</sub>O, pH 7.2) and ISP-2 medium. For antimicrobial activity, the isolate was inoculated on plates and incubated at 28 °C for 7 days. Phytopathogenic bacteria were inoculated onto Luria Agar medium plates and inoculated by agar block (7 mm diameter) in the center of the Petri dish, then incubated at 30 °C for 24 hours. The isolate was screened for antifungal activity by inoculating a single streak of Cq 72-27 in the middle of the DNPM and ISP-2 medium plate. The plates were incubated for 4 days at 28 °C and seeded with phytopathogenic fungi on



**Figure 1.** Phylogenetic trees (NJ) based on 16S rRNA gene sequences (a) and MLSA analysis (b), showing the evolutionary interactions of strain Cq 72–27 with related species of the genus *Amycolatopsis*. The sequence of the 16S rRNA of *Pseudonocardia antarctica* DVS 5a1 was used for rooting the tree. Strains that produce antibiotics are marked with an asterisk

the edges of plates, and finally incubated for 3–4 days at 28 °C. The microbial interactions were analyzed by measuring the distance of inhibition (mm).

## 2.6 Growth test

Seeds of wheat were surface sterilized with 3% sodium hypochlorite for 30 min and 70% ethanol for 15 min, then rinsed thoroughly in sterile distilled water. For the test, ten seeds were transferred to ISP-2 plates (control) or plates with ISP-2 inoculated with the isolate Cq 72-27 for 7 days. Plates were incubated at 22 °C and 4 °C for 7 days. Individual seedlings' root and shoot lengths were measured to determine the growth parameters. Five plants from each experiment variant were selected, and the experiment was repeated three times. ANOVA test was used for statistical analysis. All statistical calculations were performed in the Microsoft Office software.

## 3 Results

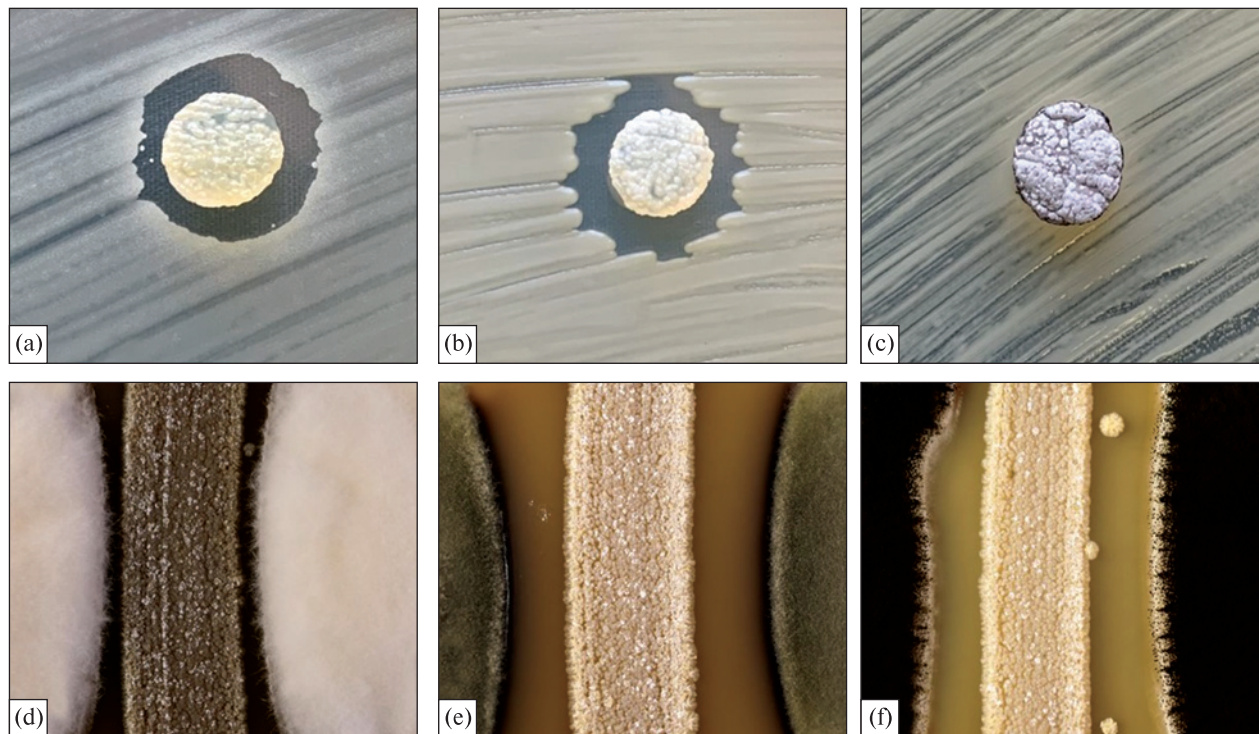
### 3.1 Phylogenetic analysis of the isolate

The 16S rRNA gene sequence of isolate Cq 72-27 was compared with all available nucleotide sequences

of other closely related *Amycolatopsis* species using the NCBI database. The data showed the most sequence similarity with three *Amycolatopsis* spp., i.e., *A. saalfeldensis* 2410-017 (97.81%), *A. xylanica* strain TU20 (97.42%), and *A. nigrescens* strain CSC17Ta-90 (97.04%). In the phylogenetic tree constructed using the neighbor-joining method, isolate Cq 72-27 formed a clade with *A. xylanica* strain DSM 45285 (Fig. 1a). Based on the observation that the bootstrap value exceeds 50%, it suggests the possibility that the isolate could pertain to a novel species. Nonetheless, it is crucial to note that genetic information alone is insufficient for the definitive identification of a new species. MLSA analysis shows that the isolate does not form a common clade with any strain, but it is close to the clade of the vancoresmycin-producing strain *A. vancoresmycina* NRRL B-24208 (Fig. 1b).

### 3.2 Cultural properties

*Amycolatopsis* sp. Cq 72-27 grew well in all tested media. The strain grew best on the ISP-2 medium, on which it formed ivory colonies. This strain could grow from 10 °C to 30 °C (optimum 28 °C). The isolate



**Figure 2.** Antagonistic effect of the strain *Amycolatopsis* sp. Cq 72-27 on phytopathogenic bacteria and fungi. (a–c) – block with the strain used for the study; (d–f) – strain is inoculated with a stroke between two points of fungal growth. (a) – *Bacillus subtilis* ATCC 31324, (b) – *Xanthomonas campestris* pv. *campestris* IMB8003, (c) – *Pseudomonas syringae* IMB 8511, (d) – *Fusarium oxysporum* IMB 54201, (e) – *Alternaria alternata* DSM 1102, (f) – *Aspergillus niger* IMB 16706

could grow in the presence of 2.5% NaCl as well as in the presence of 5 % Na<sub>2</sub>SO<sub>4</sub>. The isolate grew well between pH 4 and 12, with optimal growth at pH 7.0.

### 3.3 Antimicrobial activity

The strain *Amycolatopsis* sp. Cq 72-27 was tested for its ability to inhibit the growth of phytopathogenic bacteria and fungi. The strain showed activity against *X. campestris* pv. *campestris* IMB8003 and *Bacillus subtilis* ATCC 31324 on the ISP-2 medium. On the DNPM medium, the strain showed fungicidal activity against all tested phytopathogenic fungi (Fig. 2).

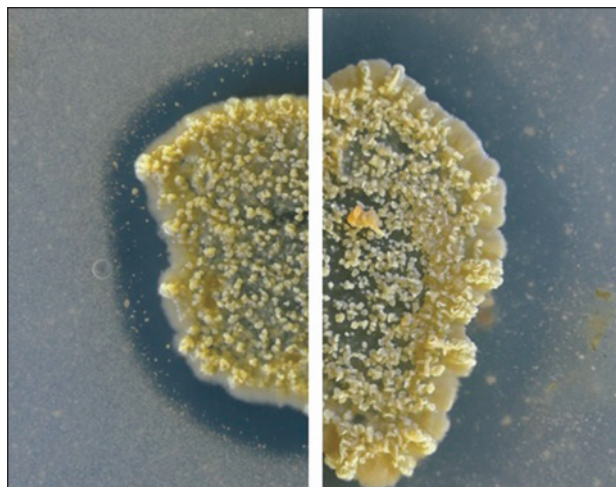
### 3.4 PGP activities and influence on seedling growth

The *Amycolatopsis* sp. Cq 72-27 does not synthesize IAA and siderophores but is capable of solubilizing phosphorus and zinc (Fig. 3), and is also involved in nitro-

gen metabolism. This strain can metabolize nitrates to nitrites and ammonia; this process is followed by the release of excess of these compounds into the medium. These properties can improve the mineral nutrition of plants, which in turn can affect plant growth.

To evaluate the stimulatory effect of *Amycolatopsis* sp. Cq 72-27, we studied the germination of wheat seeds inoculated with spores of this strain at two temperature conditions: 4 and 22 °C. At low air temperature, we did not observe any visible differences in the germination of control and inoculated seeds. Meanwhile, at 22 °C, the length of the roots formed by inoculated seeds was  $4.48 \pm 0.26$  cm, 50% longer than the roots of control seeds. Also, the height of the coleoptile increased significantly (almost twice) from  $1.2 \pm 0.1$  in control to  $2.3 \pm 0.12$  cm in inoculated seeds (Fig. 4).

The results suggest that the strain may play an important role in the adaptation of plants to unfavor-



**Figure 3.** Areas of clarity on a white background indicate the solubilization of zinc (left) and phosphorus (right) by the strain *Amycolatopsis* sp. Cq 72-27

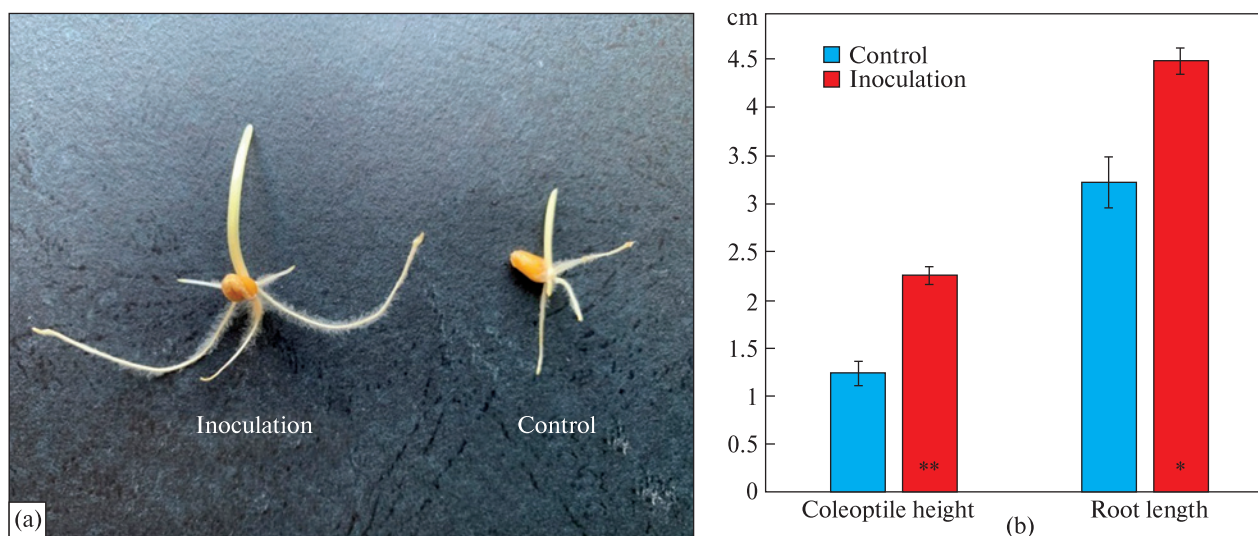
able environmental conditions, including in the Antarctic, in particular, by improving mineral nutrition.

#### 4 Discussion

*Amycolatopsis* is a rare genus of the *Pseudonocardiaceae* family which includes 86 species (<http://www.bacterio.net/amycolatopsis.html>). Members of

this genus produce bioactive compounds such as antibacterial (vancomycin, rifampicin, kigamicins, chloroorienticins), antifungal (echinosporin, 7-deoxyechinosporin), and anticancer substances (tetranogomycin, amycolactam) (Li et al., 2021). *Amycolatopsis* colonizes various soil and aquatic habitats and can also be found in other living organisms (sponges, lichens, plants, and insects) (Song et al., 2021). At present, we know antarctic *Amycolatopsis* isolated from the surface of the brown macro-alga and later classified as a new species *A. antarctica* (Wang et al., 2018); however, the strain Cq 72-27 is the first Antarctic rhizospheric member of the genus *Amycolatopsis*.

Notably, in many screening research programs focused on antarctic culturable actinomycetes, there are few reports of *Pseudonocardiaceae*. The new species of *Pseudonocardia antarctica* DVS 5a1 from a moraine in the McMurdo Dry Valleys (Prabakar et al., 2004), as well as members of the genus *Umezawaea* from the rhizosphere of *Deschampsia antarctica* É. Desv. (Galindez Island) (Tistechok et al., 2021) are significant. Given the few reports on Antarctic members of both the genus *Amycolatopsis* and the entire family of *Pseudonocardiaceae*, it can be assumed that these organisms are not very common in this

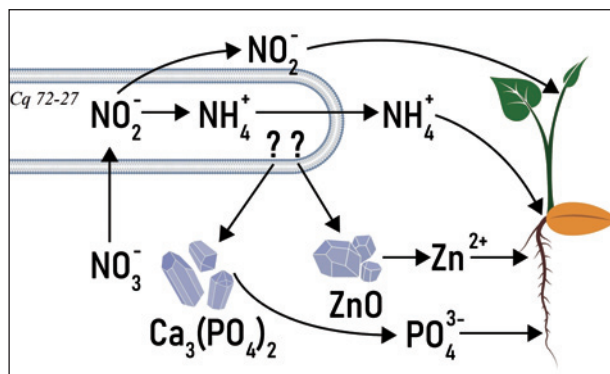


**Figure 4.** General appearance (a) and the growth parameters (b) of wheat seedlings at 22 °C after inoculation with the strain *Amycolatopsis* sp. Cq 72-27. Measurements were made on the seventh day of plant growth. Significantly different from the control for  $p < 0.05$  (\*),  $p < 0.01$  (\*\*)

area or are difficult to cultivate *in vitro*. Thus, the strain *Amycolatopsis* sp. Cq 72-27 will be valuable for studying the adaptations to adverse conditions within the genus *Amycolatopsis*. In addition, the fact that both strains *Amycolatopsis* sp. Cq 72-27 and *A. antarctica* were associated with plants may indicate complex inter-organism interactions, which, among other things, provide better adaptation to the adverse conditions of Antarctica.

Since the minimum temperature for strain growth was +10 °C, we suggested that the strain has a short growing period during December–March because at this time, the temperature on Booth Island is the highest. However, because of the periods with extremely low temperatures typical for the Antarctic, we assume that the strain can effectively survive temperature changes, which may give it an advantage over other bacteria used as biofertilizers. As the strain could also develop in a broad pH range (4–12), it can grow in the rhizosphere of different plants and in different substrates, making it attractive for use as a universal biofertilizer. Another characteristic of the strain is its ability to grow in the presence of sodium chloride and sodium sulfate, which cause soil pollution (Ivushkin et al., 2019). Although there are no direct data on the role of this strain in plant resistance to salinity, it may increase plant resistance to salt stress by producing osmoprotective metabolites (Sévin et al., 2016).

As mentioned earlier, some members of *Amycolatopsis* produce numerous biologically active substances, and the tested isolate was no exception. Thus, strain *Amycolatopsis* sp. Cq 72-27 inhibited the growth of both phytopathogenic bacteria and fungi. On the ISP-2 medium, the strain inhibited only phytopathogenic bacteria, while on the DNPM medium, it had strong fungicidal activity. We suggest that the components of the medium are crucial for activating the synthesis of secondary metabolites, so yeast cell fragments (which are part of the DNPM medium) act as elicitors of fungicidal activity. There is evidence that the addition of baker's yeast to the medium increased the synthesis of the antifungal compound rimocidin in *Streptomyces rimosus* M527 (Song et al., 2020), and a similar effect can be observed in *Amycolatopsis*



**Figure 5.** Schematic representation of the PGP properties of the strain *Amycolatopsis* sp. Cq 72-27

sp. Cq 72-27. It is also possible that the chitin from the yeast activates chitinase, which has strong fungicidal properties (Loc et al., 2020). The activity against bacteria may be related to the functioning of genes that can be activated or inactivated in a maltose-rich medium such as the ISP-2 medium (Zong et al., 2022). In addition, maltose is found in many plant organs, especially in seeds, and can be an important component of plant-bacterial interactions. This may have a direct or indirect impact on the involvement of actinomycetes in the rhizosphere of Antarctic plants as antagonists of phytopathogenic microbiota and promotion of plants' protection. In consideration of this, the strain is promising as a biocontrol agent for plant diseases of both bacterial and fungal origin.

Although the strain *Amycolatopsis* sp. Cq 72-27 has no direct effect on plant growth via the synthesis of phytohormones, it can affect plants indirectly by improving mineral nutrition. Mineral nutrients often form insoluble forms in the soil that are not available to plants. The ability to solubilize phosphorus and zinc can improve the physiological state of plants. In particular, improved phosphorus supply can enhance photosynthesis and cell division (Rawat et al., 2021), while zinc stimulates plant resistance to stress factors (Umair et al., 2020).

Nitrogen, which plays one of the most important roles in the mineral nutrition of plants, deserves special attention. The main form of nitrogen supply is inorganic forms; some plants can fix atmospheric nitrogen, as well as organic forms. Moreover, although

plants can assimilate both nitrates and ammonia, with the ammonia nutrition, the nitrogen content in plant organs is higher (Guo et al., 2019). The strain *Amycolatopsis* sp. Cq 72-27 forms nitrites and ammonia from nitrates, which would help to improve nitrogen supply. In addition, the ability to form ammonia indicates the potential ability to mineralize organic forms of nitrogen, making them available to plants. Reducing nitrates can decrease the environmental damage that occurs when using mineral fertilizers which causes water pollution (Bijay-Singh & Craswell, 2021). Consequently, the strain can become the basis of biofertilizers since it can improve the mineral nutrition of plants (Fig. 5).

This can be important for plant development, especially on poor soils. Such properties can be crucial in poor antarctic soils with high insoluble mineral content. This affects the associated plants and the entire microbiome, as phosphorus, zinc, and other minerals are part of many macromolecules that perform important functions in living organisms.

Microorganisms play an important role in plant life; they not only improve mineral nutrition but also take part in the formation of plant resistance to stress, both biotic and abiotic (Khanna et al., 2019; Vaishnav et al., 2019). However, not enough information is available about the impact of antarctic bacteria on associated plants. Antarctic bacteria can increase plant resistance to salt (Gallardo-Cerda et al., 2018) and cold stress (Yarzabal et al., 2018). The tested strain increased the growth of inoculated seedlings almost twice at 22 °C, which may have an important impact during germination and competition for resources. In addition, the strain's cold tolerance may benefit the inoculated plants during the cold season. The results suggest a potentially positive effect on plant growth and development, confirming the strain's potential as a component of biological fertilizer. This strain can be a platform for creating biological products that can function at low temperatures and in different soil types.

## 5 Conclusions

This study focuses on the PGP properties of the Antarctic strain *Amycolatopsis* sp. Cq 72-27. We found

that the strain can affect the supply of minerals to plants, as it can metabolize nitrates and solubilize zinc and phosphorus. In addition, we showed a significant effect on the growth of wheat seedlings. We believe the strain under study can become a biofertilizer base and find wide application in plant growth promotion.

*Author contributions.* Conceptualization: I.R. and O.G.; investigation: I.R.; writing-original draft preparation: I.R.; writing-review and editing: O.G.; supervision: O.G. Both authors have read and agreed to the final version of the manuscript.

*Funding.* This study was supported by grant H/309-2003 from the Ministry of Education and Science of Ukraine and partially funded by grant H/04-2021 of the State Institution National Antarctic Scientific Center.

*Conflict of Interest.* The authors declare that they have no conflict of interest.

## References

- Bhakat, K., Chakraborty, A., & Islam, E. (2021). Characterization of zinc solubilization potential of arsenic tolerant *Burkholderia* spp. isolated from rice rhizospheric soil. *World Journal of Microbiology and Biotechnology*, 37(3), 39. <https://doi.org/10.1007/s11274-021-03003-8>
- Bijay-Singh, & Craswell, E. (2021). Fertilizers and nitrate pollution of surface and ground water: an increasingly pervasive global problem. *SN Applied Sciences*, 3(4), 518. <https://doi.org/10.1007/s42452-021-04521-8>
- Cueva-Yesquén, L. G., Goulart, M. C., Attili de Angelis, D., Nopper Alves, M., & Fantinatti-Garborgini, F. (2021). Multiple plant growth-promotion traits in endophytic bacteria retrieved in the vegetative stage from passionflower. *Frontiers in Plant Science*, 11, 621740. <https://doi.org/10.3389/fpls.2020.621740>
- French, E., Kaplan, I., Iyer-Pascuzzi, A., Nakatsu, C. H., & Enders, L. (2021). Emerging strategies for precision microbiome management in diverse agroecosystems. *Nature Plants*, 7(3), 256–267. <https://doi.org/10.1038/s41477-020-00830-9>
- Gallardo-Cerda, J., Levihuan, J., Lavín, P., Osés, R., Atala, C., Torres-Díaz, C., Cuba-Díaz, M., Barrera, A., & Molina-Montenegro, M. A. (2018). Antarctic rhizobacteria improve salt tolerance and physiological performance of the Antarctic vascular plants. *Polar Biology*, 41(10), 1973–1982. <https://doi.org/10.1007/s00300-018-2336-z>
- Glaeser, S. P., & Kämpfer, P. (2015). Multilocus sequence analysis (MLSA) in prokaryotic taxonomy. *Systematic and applied microbiology*, 38(4), 237–245. <https://doi.org/10.1016/j.syapm.2015.03.007>



- Gohain, A., Manpoong, C., Saikia, R., & De Mandal, S. (2020). Actinobacteria: Diversity and biotechnological applications. In S. de Mandal, & P. Bhatt (Eds.), *Recent Advances in Microbial Diversity* (1st ed., pp. 217–231). <https://doi.org/10.1016/B978-0-12-821265-3.00009-8>
- Guo, J., Jia, Y., Chen, H., Zhang, L., Yang, J., Zhang, J., Hu, X., Ye, X., Li, Y., & Zhou, Y. (2019). Growth, photosynthesis, and nutrient uptake in wheat are affected by differences in nitrogen levels and forms and potassium supply. *Scientific Reports*, *9*(1), 1248. <https://doi.org/10.1038/s41598-018-37838-3>
- Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, *51*, 72–80. <https://doi.org/10.1016/j.mib.2019.10.008>
- Igarashi, Y. (2004). Screening of novel bioactive compounds from plant-associated actinomycetes. *Actinomycetologica*, *18*(2), 63–66. [https://doi.org/10.3209/saj.18\\_63](https://doi.org/10.3209/saj.18_63)
- Ivushkin, K., Bartholomeus, H., Bregt, A. K., Pulatov, A., Kempen, B., & De Sousa, L. (2019). Global mapping of soil salinity change. *Remote Sensing of Environment*, *231*, 111260. <https://doi.org/10.1016/j.rse.2019.111260>
- Khanna, K., Jamwal, V. L., Kohli, S. K., Gandhi, S. G., Ohri, P., Bhardwaj, R., Wijaya, L., Nasser Alyemini, M., & Ahmad, P. (2019). Role of plant growth promoting Bacteria (PGPRs) as biocontrol agents of *Meloidogyne incognita* through improved plant defense of *Lycopersicon esculentum*. *Plant and Soil*, *436*(1), 325–345. <https://doi.org/10.1007/s11104-019-03932-2>
- Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., & Hopwood, D. A. (2000). *Practical streptomyces genetics* (Vol. 291). John Innes Foundation.
- Kumar, A., Singh, S., Gaurav, A. K., Srivastava, S., & Verma, J. P. (2020). Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. *Frontiers in Microbiology*, *11*, 1216. <https://doi.org/10.3389/fmicb.2020.01216>
- Li, R., Wang, M., Ren, Z., Ji, Y., Yin, M., Zhou, H., & Tang, S. K. (2021). *Amycolatopsis ailingensis* sp. nov., a halotolerant actinobacterium, produces new secondary metabolites. *Frontiers in Microbiology*, *12*, 743116. <https://doi.org/10.3389/fmicb.2021.743116>
- Loc, N. H., Huy, N. D., Quang, H. T., Lan, T. T., & Thu Ha, T. T. (2020). Characterisation and antifungal activity of extracellular chitinase from a biocontrol fungus, *Trichoderma asperellum* PQ34. *Mycology*, *11*(1), 38–48. <https://doi.org/10.1080/21501203.2019.1703839>
- Louden, B. C., Haarmann, D., & Lynne, A. M. (2011). Use of blue agar CAS assay for siderophore detection. *Journal of Microbiology & Biology Education*, *12*(1), 51–53. <https://doi.org/10.1128/jmbe.v12i1.249>
- Majeed, A., Muhammad, Z., & Ahmad, H. (2018). Plant growth promoting bacteria: role in soil improvement, abiotic and biotic stress management of crops. *Plant Cell Reports*, *37*(12), 1599–1609. <https://doi.org/10.1007/s00299-018-2341-2>
- Martínez-Romero, E., Aguirre-Noyola, J. L., Taco-Taype, N., Martínez-Romero, J., & Zuñiga-Dávila, D. (2020). Plant microbiota modified by plant domestication. *Systematic and Applied Microbiology*, *43*(5), 126106. <https://doi.org/10.1016/j.syapm.2020.126106>
- Notredame, C., Higgins, D. G., & Heringa, J. (2000). T-Coffee: A novel method for fast and accurate multiple sequence alignment. *Journal of Molecular Biology*, *302*(1), 205–217. <https://doi.org/10.1006/jmbi.2000.4042>
- Núñez-Montero, K., & Barrientos, L. (2018). Advances in Antarctic research for antimicrobial discovery: a comprehensive narrative review of bacteria from Antarctic environments as potential sources of novel antibiotic compounds against human pathogens and microorganisms of industrial importance. *Antibiotics*, *7*(4), 90. <https://doi.org/10.3390/antibiotics7040090>
- Prabhar, V., Dube, S., Reddy, G. S. N., & Shivaji, S. (2004). *Pseudonocardia antarctica* sp. nov. an Actinomycetes from McMurdo Dry Valleys, Antarctica. *Systematic and Applied Microbiology*, *27*(1), 66–71. <https://doi.org/10.1078/0723-2020-00249>
- Rawat, P., Das, S., Shankhdhar, D., & Shankhdhar, S. C. (2021). Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *Journal of Soil Science and Plant Nutrition*, *21*(1), 49–68. <https://doi.org/10.1007/s42729-020-00342-7>
- Rihan, H. Z., Al-Issawi, M., & Fuller, M. P. (2017). Advances in physiological and molecular aspects of plant cold tolerance. *Journal of Plant Interactions*, *12*(1), 143–157. <https://doi.org/10.1080/17429145.2017.1308568>
- Sathya, A., Vijayabharathi, R., & Gopalakrishnan, S. (2017). Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. *3 Biotech*, *7*(2), 102. <https://doi.org/10.1007/s13205-017-0736-3>
- Sévin, D. C., Stählin, J. N., Pollak, G. R., Kuehne, A., & Sauer, U. (2016). Global metabolic responses to salt stress in fifteen species. *PLoS One*, *11*(2), e0148888. <https://doi.org/10.1371/journal.pone.0148888>
- Shekhar Nautiyal, C. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, *170*(1), 265–270. <https://doi.org/10.1111/j.1574-6968.1999.tb13383.x>
- Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of Streptomyces species. *International Journal of Systematic Bacteriology*, *16*(3), 313–340.
- Singh, J. P. (1988). A rapid method for determination of nitrate in soil and plant extracts. *Plant and Soil*, *110*(1), 137–139. <https://doi.org/10.1007/BF02143549>
- Singh, R., & Dubey, A. K. (2018). Diversity and applications of endophytic actinobacteria of plants in special and other ecological niches. *Frontiers in Microbiology*, *9*, 1767. <https://doi.org/10.3389/fmicb.2018.01767>

- Song, Z., Ma, Z., Bechthold, A., & Yu, X. (2020). Effects of addition of elicitors on rimocidin biosynthesis in *Streptomyces rimosus* M527. *Applied Microbiology and Biotechnology*, 104(10), 4445–4455. <https://doi.org/10.1007/s00253-020-10565-4>
- Song, Z., Xu, T., Wang, J., Hou, Y., Liu, C., Liu, S., & Wu, S. (2021). Secondary metabolites of the genus *Amycolatopsis*: Structures, bioactivities and biosynthesis. *Molecules*, 26(7), 1884. <https://doi.org/10.3390/molecules26071884>
- Styczynski, M., Bieganski, G., Decewicz, P., Rewerski, B., Debiec-Andrzejewska, K., & Dziewit, L. (2022). Application of psychrotolerant antarctic bacteria and their metabolites as efficient plant growth promoting agents. *Frontiers in Bioengineering and Biotechnology*, 10. <https://doi.org/10.3389/fbioe.2022.772891>
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Tirry, N., Joutey, N. T., Sayel, H., Kouchou, A., Bahafid, W., Asri, M., & El Ghachtouli, N. (2018). Screening of plant growth promoting traits in heavy metals resistant bacteria: prospects in phytoremediation. *Journal of Genetic Engineering and Biotechnology*, 16(2), 613–619. <https://doi.org/10.1016/j.jgeb.2018.06.004>
- Tistechok, S., Skvortsova, M., Luzhetskyy, A., Fedorenko, V., Parnikoza, I., & Gromyko, O. (2019). Antagonistic and plant growth promoting properties of actinomycetes from rhizosphere *Deschampsia antarctica* È. Desv. (Galindez Island, Antarctica). *Ukrainian Antarctic Journal*, 1(18), 169–177. [https://doi.org/10.33275/1727-7485.1\(18\).2019.140](https://doi.org/10.33275/1727-7485.1(18).2019.140)
- Tistechok, S., Skvortsova, M., Mytsyk, Y., Fedorenko, V., Parnikoza, I., Luzhetskyy, A., & Gromyko, O. (2021). The diversity and antibacterial activity of culturable actinobacteria isolated from the rhizosphere soil of *Deschampsia antarctica* (Galindez Island, Maritime Antarctic). *Polar Biology*, 44(9), 1859–1868. <https://doi.org/10.1007/s00300-021-02924-2>
- Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L., Nawaz, M., Rasheed, A., Afzal, A., Liu, Y., & Guoqin, H. (2020). The critical role of zinc in plants facing the drought stress. *Agriculture*, 10(9), 396. <https://doi.org/10.3390/agriculture10090396>
- Vaishnav, A., Shukla, A. K., Sharma, A., Kumar, R., & Choudhary, D. K. (2019). Endophytic bacteria in plant salt stress tolerance: current and future prospects. *Journal of Plant Growth Regulation*, 38(2), 650–668. <https://doi.org/10.1007/s00344-018-9880-1>
- Wang, J., Leiva, S., Huang, J., & Huang, Y. (2018). *Amycolatopsis antarctica* sp. nov., isolated from the surface of an Antarctic brown macroalga. *International Journal of Systematic and Evolutionary Microbiology*, 68(7), 2348–2356. <https://doi.org/10.1099/ijsem.0.002844>
- Yamal, G., Sharmila, P., Rao, K. S., & Pardha-Saradhi, P. (2013). Yeast Extract Mannitol medium and its constituents promote synthesis of Au nanoparticles. *Process Biochemistry*, 48(3), 532–538. <https://doi.org/10.1016/j.procbio.2013.02.011>
- Yarzabal, L. A., Monserrate, L., Buela, L., & Chica, E. (2018). Antarctic *Pseudomonas* spp. promote wheat germination and growth at low temperatures. *Polar Biology*, 41(11), 2343–2354. <https://doi.org/10.1007/s00300-018-2374-6>
- Zhao, Y., Shi, R., Bian, X., Zhou, C., Zhao, Y., Zhang, S., Wu, F., Waterhouse, G. I. N., Wu, L.-Z., Tung, C.-H., & Zhang, T. (2019). Ammonia detection methods in photocatalytic and electrocatalytic experiments: how to improve the reliability of NH<sub>3</sub> production rates? *Advanced Science*, 6(8), 1802109. <https://doi.org/10.1002/advs.201802109>
- Zong, G., Fu, J., Zhang, P., Zhang, W., Xu, Y., Cao, G., & Zhang, R. (2022). Use of elicitors to enhance or activate the antibiotic production in *streptomyces*. *Critical Reviews in Biotechnology*, 42(8), 1260–1283. <https://doi.org/10.1080/07388551.2021.1987856>

Received: 19 April 2023  
Accepted: 20 July 2023

## I. Роман, О. Громико\*

Львівський національний університет імені Івана Франка,  
м. Львів, 79005, Україна

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

### Фітостимульовальні властивості антарктичного штаму *Amycolatopsis* sp. Cq 72-27

**Реферат.** Малодосліджені біотопи можуть бути джерелом фітостимульовальних бактерій з унікальними властивостями. Оскільки Антарктика характеризується екстремальними умовами, то ця територія є перспективною для дослідження різних груп мікроорганізмів, зокрема й фітостимульовальних. Ми вважаємо, що в складних умовах Антарктиди бактерії могли отримати пристосування, які будуть цінні й для використання їх у сільському господарстві. Для дослідження властивостей антарктичного ізоляту нами було використано низку методів, а саме: молекулярні, для

встановлення нуклеотидної послідовності генів; мікробіологічні, для дослідження культуральних властивостей, а також фітостимулювальних та антимікробних властивостей штаму. Штам Cq 72-27 належить до роду *Amycolatopsis*, він виявляв антагоністичний ефект проти фітопатогенних бактерій (*Xanthomonas campestris* pv. *campestris* IMB8003 та *Bacillus subtilis* ATCC 31324) та грибів (*Alternaria alternata* DSM 1102, *Fusarium oxysporum* IMB 54201 та *Aspergillus niger* IMB 16706). Крім того, штам Cq 72-27 солюбілізував фосфор і цинк та утворював нітрити й аміак. Інокуляція насіння пшениці його спорами сприяла проростанню та росту сіянців *in vitro*. Штам *Amycolatopsis* sp. Cq 72-27 може стати основою для біодобрив, оскільки продемонстрував здатність покращувати мінеральне живлення рослин та пригнічувати ріст фітопатогенних мікроорганізмів.

**Ключові слова:** антарктичні актиноміцети, біоконтроль, ризосферні бактерії, фітостимулювальні бактерії