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ADAPTIVE AND BIOTECHNOLOGICAL POTENTIAL OF *STREPTOMYCES* STRAINS ISOLATED FROM ADVERSE BIOTOPES

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Background. *Streptomyces* spp. from adverse biotopes are characterized by broad adaptive capacities, unique metabolism, and biotechnological potential. In this study, *Streptomyces* strains isolated from adverse habitats were investigated through phylogenetic analysis, bioactivity assays, and evaluation of their adaptive and biotechnological potential.

Materials and Methods. Genetic methods (DNA extraction and 16S rRNA gene sequencing), microbiological methods (agar block confrontation assay for antagonism; cultivation on nutrient media supplemented with heavy metals to assess metal resistance; Kirby–Bauer disk diffusion method for antibiotic susceptibility testing), biochemical methods (enzymatic activity assays and carbon source utilization), and bioinformatic tools (molecular phylogeny in MEGA12.1.1; genome annotation using RAST and DFAST) were employed.

Results. Phylogenetic analysis revealed close relatedness among strains 89, 34, and SK7, which clustered into a single clade of the phylogenetic tree. They exhibited moderate antifungal activity. Among them, strain 89 inhibited fungi of the genera *Fusarium* and *Rhizoctonia*. Chromium and iron were the least toxic to all strains; zinc and cobalt showed moderate toxicity, while nickel and copper were the most toxic. Growth inhibition occurred at 25 mg/L of nickel and 50 mg/L of copper; only strain 89 tolerated 100 mg/L of copper. Strain 89 exhibited lipase, amylase, and cellulase activities, along with the ability to hydrolyze casein and metabolize glycerol, mannitol, and diverse sugars, thereby underscoring its potential for the degradation of organic residues.



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Conclusions. *S. anulatus* 89 possesses broad adaptive and biotechnological potential and inhibits some phytopathogens.

Keywords: *Streptomyces*, adverse biotopes, antagonism, phytopathogens, heavy metals, resistance, enzymatic activity

ABBREVIATIONS

DFAST – DDBJ Fast Annotation and Submission Tool

RAST – Rapid Annotation using Subsystem Technology

PDA – potato dextrose agar

GYM – glucose-yeast extract-malt extract nutrient medium

PGA – potato glucose medium

SAA – starch ammonia agar

BGC – biosynthetic gene cluster

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INTRODUCTION

Streptomyces spp. are known as producers of the wide range of natural products that have applications in many fields (Lasch *et al.*, 2026). However, in recent decades, the re-discovery of previously known compounds has become increasingly common, leading to active research of strains isolated from non-optimal environments, for example, saline, alkaline, arid, and contaminated soils, etc. To survive in such conditions, they developed special adaptations, including the secretion of siderophores that chelate toxic ions, the synthesis of melanin-like pigments to mitigate oxidative damage, and intracellular sequestration through metallothioneins, histidines, or specialized storage proteins (Djinni & Djoudi, 2022). Such strains often possess unique biosynthetic potential. For example, extremophilic *Streptomyces* spp. from the Atacama Desert, have yielded novel bioactive compounds with antibacterial and anticancer properties, including Abenquines A-D, Atacamycins A-C, Chaxalactins A-C, Chaxamycins A-D, etc. Beyond pharmaceuticals, their metabolites, such as pigments and osmoprotectants, hold significant industrial value (Han *et al.*, 2024). The antifungal properties of *Streptomyces* spp. are highly important for agriculture, as farmland is frequently infected by phytopathogens. Representatives of the genera *Fusarium* and *Rhizoctonia* are among the most destructive, causing root rot, wilting, and substantial yield losses in cereals and other crops, while also reducing grain quality (Fang *et al.*, 2026). Bioactive soil streptomycetes, resistant to heavy metals, can exert growth-promoting and stress-protective effects on plants in harsh conditions (Norte *et al.*, 2025). They are recognized for their ability to enhance heavy metal extraction by phytoremediants, suppress phytopathogens, promote plant growth and development, and improve antioxidant status and stress resilience (Ali *et al.*, 2017; Ye *et al.*, 2026). Although a significant proportion of research has focused on obligate extremophiles, there is a significant gap in the knowledge regarding stress-tolerant strains with high ecological plasticity. Unlike obligate extremophiles, such adaptive strains can actively colonize ordinary agricultural soils, whilst retaining the ability to survive sudden environmental shocks, such as fluctuations in salinity or severe heavy metal contamination (Norte *et al.*, 2025). For Ukraine, the isolation and investigation of the adaptive and biosynthetic potential of *Streptomyces* strains is of particular importance in light of

the ongoing military actions, which have resulted in the accumulation of heavy metals in agricultural soils, the destruction of microbial biodiversity, and the spread of phytopathogens, further aggravated by climate changes (Solokha *et al.*, 2024). The identification of bioactive microorganisms, resistant to heavy metals, may therefore hold promise for future applications, particularly in soil bioremediation biotechnology. The evaluation of promising *Streptomyces* strains requires a comprehensive assessment of their evolutionary relationships, adaptive potential, bioactivity, resistance to abiotic stresses, and cultivation parameters. It has previously been confirmed that *S. anulatus* 89, isolated from a saline environment, produces a range of anticancer antibiotics and induces early apoptosis in cancer cell lines, whereas its bioactivity against phytopathogens and adaptive potential have not been investigated. Therefore, the study aimed to screen *Streptomyces* strains from adverse biotopes and investigate their adaptive and biotechnological potential. Accordingly, the study included the determination of evolutionary relationships of the studied strains, their antagonistic properties, enzymatic activity, resistance to heavy metals, and the ability to grow under NaCl concentrations. The main hypothesis was that strains isolated from adverse biotopes possess broad adaptive potential and may represent valuable resources for biotechnology.

MATERIALS AND METHODS

Sampling locations. *Streptomyces* strains were previously isolated from adverse environments, namely strain 89 – from the coastal zone of the Mykolaiv region, Ukraine (46.8 N, 31.9°E) (Sylchuk *et al.*, 2026), strain 34 – from the Dobrich region of Bulgaria in the saline coastal area of the Black Sea, and strain SK7 – from soil in Kryvyi Rih, Ukraine, collected near “Kryvorizhstal” (Kryvyi Rih, 47°28'26.53"N, 33°38'57.62"E), a territory impacted by a ballistic missile. Their genomes encode a large number of biosynthetic gene clusters (BGCs), particularly those isolated from extreme environments, are valuable sources of bioactive compounds. The whole genome and the 16S rRNA gene sequences of strain 89 (GCA_053710995.1, PZ352888), together with the 16S rRNA sequences of the strains 34 and SK7 (PZ352890 and PZ352889, respectively) were deposited in GenBank. The whole genome annotation of *S. anulatus* 89 was described previously (Sylchuk *et al.*, 2026).

Cultivation of *Streptomyces* strains. *Streptomyces* strains were cultivated on potato-glucose agar (PGA – potato infusion 200 g, dextrose 20 g, agar 15 g, distilled water 1000.0 mL, and pH 5.5), glucose-yeast extract-malt extract medium (GYM – glucose 4.0 g, yeast extract 4.0 g, malt extract 10.0 g, CaCO₃ 2.0 g, agar 20.0 g, distilled water 1000.0 mL, pH 7.2), and starch-ammonia agar (SAA) as described earlier (Loboda *et al.*, 2021). Changes in pH and biomass accumulation were measured after 7 days of cultivation (Loboda *et al.*, 2021).

Phylogenetic analysis of *Streptomyces* strains and genome annotation. Biomass of *Streptomyces* strains, cultivated on GYM medium, was used for DNA isolation employing the salt precipitation method (Wilson, 2001). The 16S rRNA gene of the bacteria was amplified by PCR using 8F primers (AGAGTTTGATYMTGGCTCAG) and 1510R (TACGGYTACCTTGTTACGACTT). The PCR products were purified and then sequenced using the Sanger sequencing method at Explogen LLC (Lviv, Ukraine). Phylogenetic analysis was performed with MEGA 12.1.1 software, using the maximum likelihood algorithm (Kumar *et al.*, 2024). DFAST and RAST tools were used for the genome annotation (Elmanzalawi *et al.*, 2025, Brettin *et al.*, 2015). Antibiotic resistance

was predicted in The Comprehensive Antibiotic Resistance Database (CARD) (Alcock *et al.*, 2023). Genome analysis of biosynthetic gene clusters (BGCs) responsible for the biosynthesis of secondary metabolites was performed using the antiSMASH 8.0 tool (Blin *et al.*, 2025). To visualize the clusters organization, synteny, and sequence identity against known reference clusters, gene cluster comparison were generated using Clinker v0.0.32 (Gilchrist & Chooi, 2021).

The analysis of streptomycetes resistance to salinity conditions. To investigate the resistance of streptomycetes to habitat salinity, the strains were cultivated on GYM medium, supplemented with a NaCl gradient of 0.1 %, 2.5 %, 5 %, 7 %, 8 %, 9 %, and 10 %; their growth and sporulation were visually assessed. *Streptomyces avermitilis* IMV Ac-5015 and *Streptomyces violaceus* IMV Ac-5027, deposited in the Depository of Non-Pathogenic Microorganisms of the IMV NASU, were included for comparison.

Enzymatic activity and utilization of different carbon sources. To determine lipolytic activity, strains were grown on solid medium (NaCl 1 g, peptone 10 g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g, agar 15 g, distilled water 900 mL; pH 7.0) and supplemented with a Tween-20 solution (10 mL of Tween-20 in 90 mL distilled water). The appearance of opaque zones around colonies indicated formation of insoluble calcium salts of fatty acids, confirming lipolytic activity (Kasana *et al.*, 2008, Gopinath *et al.*, 2005). A rapid method for the Laccase activity was tested on Bennett's medium supplemented with microelements and guaiacol, where red-brown zones around colonies indicated laccase activity (Unuofin *et al.*, 2019). Tyrosinase activity was assayed on tyrosine medium with black zones around colonies, confirming tyrosinase activity (Roy *et al.*, 2014). Amylolytic activity was determined on SAA nutrient medium, followed by flooding plates with Lugol's iodine solution; clear zones indicated starch hydrolysis (Amoozegar *et al.*, 2003). Cellulolytic activity was tested on carboxymethyl cellulose medium with plates flooded with Gram's iodine solution; clear zones around colonies indicated cellulase activity (Kasana *et al.*, 2008).

Determination of streptomycetes heavy metal resistance. The resistance of the streptomycetes to heavy metals was assessed based on their ability to grow on PGA medium supplemented with heavy metals: Zn^{2+} , Cr^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Fe^{2+} (Afzal *et al.*, 2017). Thus, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, K_2CrO_4 , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ("Alfarus", Ukraine) were used as sources of the respective ions. The calculation of different concentrations was performed based on the respective metal cation. A cell suspension containing 10^8 CFU/mL was evenly spread over the surface of the nutrient medium to form a lawn. After 7 days at 28 °C, growth, sporulation, colony coloration, and pigment diffusion into the agar were analyzed. PGA without heavy metals served as the control.

The analysis of streptomycetes antagonistic activity. The antagonistic activity of streptomycetes was assessed using a modified dual block confrontation method (Sarrocchio, 2023). Briefly, agar blocks containing fungal mycelium and grown on potato dextrose agar (PDA) (Koblitz *et al.*, 2023) were placed in the center of agar plates, while blocks with streptomycetes (previously grown on GYM) were positioned at equal distances around the block of phytopathogen. The plates were incubated at 28 °C for 7 days, followed by measurement of the inhibition zones. *Bipolaris sorokiniana* TR1, *Alternaria arborensis* TR1, *Rhizoctonia solani* DSM 22880, *Aspergillus niger* ATCC 6275, and representatives of the genus *Fusarium*: *F. solani* ATCC 11712, *F. oxysporum* TR1 and DSM 841, *F. culmorum* DSM 21713, and *F. graminearum* DSM 21727 were used as test strains. Nystatin 80 U (PJSC Scientific and Production Center Borshchahivskiy

Chemical-Pharmaceutical Plant, Kyiv, Ukraine) was used as a positive control. To investigate the antagonistic activity against phytopathogenic bacteria and opportunistic microorganisms, a dual plate assay was employed (Senthilkumar *et al.*, 2021). Inhibition zones were measured against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 25922, and *Candida albicans* CECT 1392. As phytopathogenic bacteria, *Pseudomonas syringae* IMV B-1027, *Pseudomonas fluorescens* IMV 8573, *Pectobacterium carotovorum* IMV 8982, *Xanthomonas campestris* IMV 8926, and *Agrobacterium tumefaciens* IMV 8628 were used (Feeney *et al.*, 2022).

Antibiotic susceptibility test. The antibiotic sensitivity was tested using the Kirby–Bauer disc diffusion method on GYM medium (Khan *et al.*, 2019). A total of 26 antibiotics from 8 pharmacological groups were used (polyenes, aminoglycosides, penicillins, tetracyclines, fluoroquinolones, macrolides, β -lactams, and polymyxins).

Statistical analysis. All experiments were performed in triplicate ($n = 3$), and the results are expressed as the mean $\pm$ standard deviation (SD). Prior to analysis, the data were evaluated for normality and homogeneity of variances using the Shapiro–Wilk and Levene’s tests, respectively. As the data did not meet the assumptions required for parametric analysis ($P \leq 0.05$), non-parametric one-way Kruskal–Wallis analysis of variance was utilized to assess the effects of the actinomycete strain and fungal test culture on the zone of growth inhibition. For subsequent multiple pairwise comparisons, Dunn’s post-hoc test with a Bonferroni correction was applied to determine statistical significance. To quantify the magnitude of the observed differences, the effect size for the Kruskal–Wallis test was calculated using the epsilon-squared estimate (ϵ^2), where values were interpreted as indicating small ($0.01 < 0.08$), medium ($0.08 < 0.26$), or large (≥ 0.26) effects. Exact adjusted P-values are reported for all post-hoc pairwise comparisons. All statistical analyses were conducted using Python 3.11.

RESULTS

Phylogenetic analysis of *Streptomyces* strains. To determine the evolutionary relationships, a phylogenetic tree was constructed based on the 16S rRNA genes of 89, 34, and SK7 strains (Fig. 1).

All the studied strains belong to the genus *Streptomyces*, forming a common cluster with other members of this genus. Phylogenetic analysis showed that strain SK7 is in a separate evolutionary position. Meanwhile, strains 34 and 89 form clades with *S. durocortorensis* (strain RHZ10) and *S. anulatus* (strain NBRC 13369), respectively.

Cultivation of *Streptomyces* strains. Biomass accumulation and pH variation during submerged cultivation in different nutrient media were assessed (Table 1).

Following 7-day growth in three nutrient media of distinct composition, biomass yield was higher when strains 89 and SK7 were grown in liquid GYM, whereas the starch-ammonia medium supported the lowest biomass accumulation. These findings indicate that organic nutrient media are optimal for cultivating the studied *Streptomyces* strains in biotechnological processes. The highest biomass yields were detected for strains 89 and SK7 (5.6 and 6.8 g/L, respectively). Biomass accumulation of strain 34 was comparatively lower and exhibited no significant differences across the tested media. Alkalinization of the media was evident after 7 days of cultivation, which is likely associated with the biosynthesis of secondary metabolites. All strains studied were aerobic and exhibited optimal growth and sporulation at +28 °C.

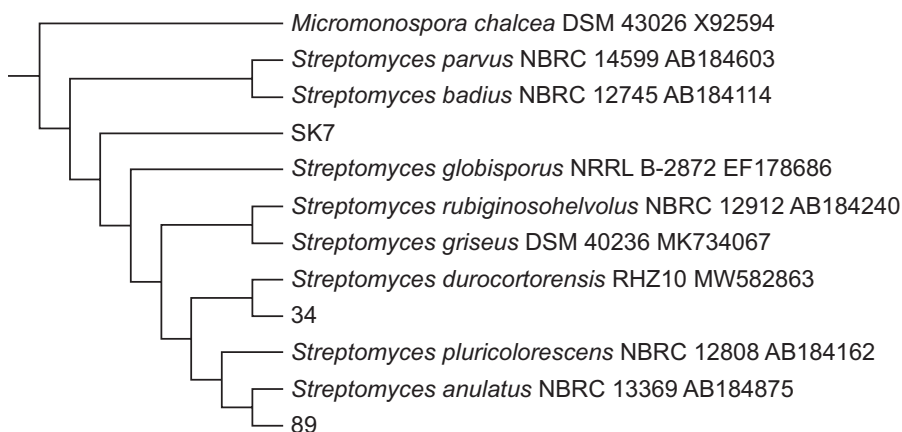


Fig. 1. Phylogenetic tree of strains 89, 34, and SK7 based on 16S rRNA gene sequences. The tree was constructed in MEGA 12.1.1 using the maximum likelihood method with 1000 bootstrap replicates. The 16S rRNA sequence of *Micromonospora chalcea* DSM 43026 was used as an outgroup

Table 1. pH variation in nutrient media during submerged cultivation of *Streptomyces* strains, with biomass accumulation ($\pm$ SD, n = 3)

Nutrient media *	Biomass, g/L	pH (initial)	pH after 7day cultivation
S. anulatus 89			
PG	4.485±0.063	7.02	7.43
GYM	5.553±0.172		7.64
SA	4.167±0.039		7.89
Streptomyces sp. SK7			
PG	5.125±0.098	7.02	7.32
GYM	6.795±0.021		7.64
SA	4.754±0.074		7.74
Streptomyces sp. 34			
PG	2.911±0.047	7.02	7.21
GYM	3.314±0.012		7.35
SA	2.657±0.074		7.57

Notes: *PG – liquid PDA without agar; GYM – liquid GYM without agar; SA – liquid SAA without agar

The analysis of streptomycetes resistance to salinity conditions. Sporulation of strains 89, 34, and SK7 was observed at a NaCl concentration of 2 %. At higher concentrations, sporulation was lost, and the maximum level supporting substrate mycelial growth was 9 %, with strain 34 exhibiting the weakest growth (**Fig. 2**). Notably, the collection strains *S. avermitilis* IMV Ac-5015 (previously isolated from soil) and *S. violaceus* IMV Ac-5027 (isolated from the same biotope as strain 34) were less tolerant to elevated NaCl concentrations. They exhibited only weak growth at 5 % NaCl, while at higher concentrations, growth was absent, with only occasional colony-forming units

observed. Strain IMV Ac-5027 produced a violet pigment; however, this trait was lost at 5 % NaCl. Thus, strains isolated from adverse biotopes are characterized by varying degrees of ecological plasticity, with strains 89, 34, and SK7 being the most tolerant and therefore assignable to the group of moderate halophiles.

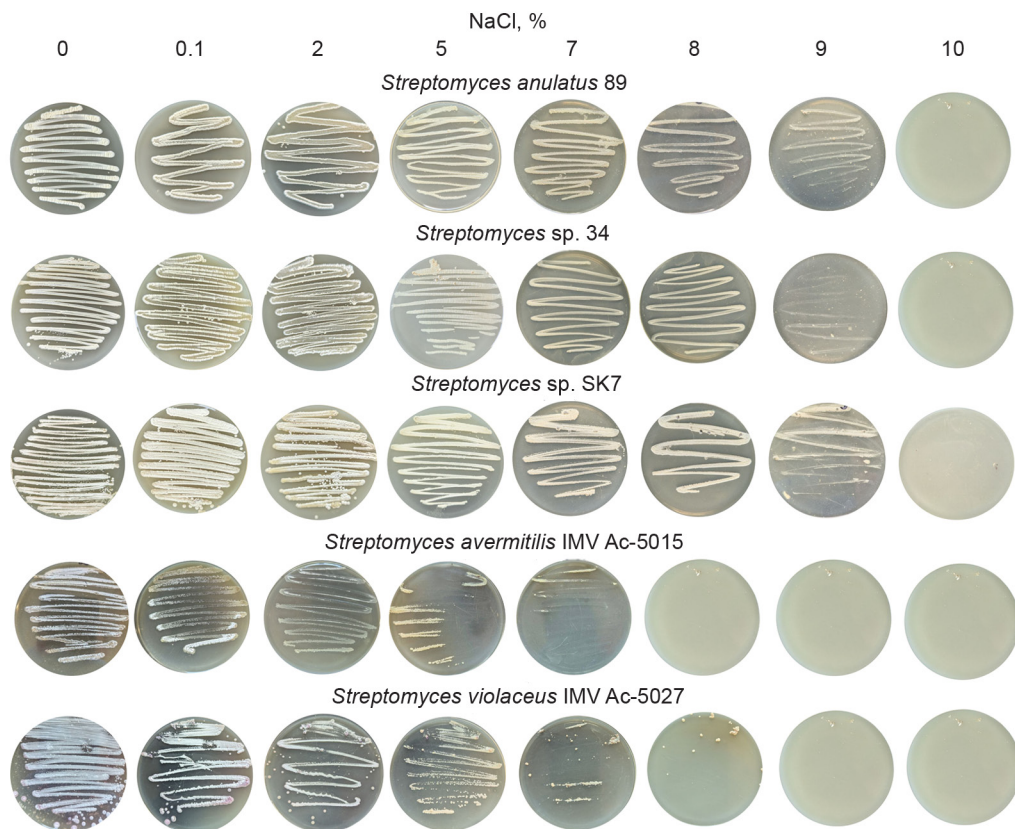


Fig. 2. Growth and sporulation of the studied *Streptomyces* strains (89, 34, and SK7) compared to the reference strains under varying NaCl concentrations (0–10 %) on GYM medium. The plates were photographed after 7 days of incubation at 28 °C

Enzymatic activity and utilization of different carbon sources. Enzymatic profile of strain 89 lacked tyrosinase activity; however, it exhibited nitrate reduction, pectin hydrolysis, casein hydrolysis, utilization of Tween 20, 40, 60, and 80, as well as, laccase, amylase, and cellulase activities. The enzymatic activity of strain 34 was identical except for laccase. Strain SK7 exhibited hydrolysis of cellulose, Tween 20, 40, 60, and 80, pectin hydrolysis, casein hydrolysis. Strains 89 and 34 utilized a wide range of carbon sources, including glycerol, mannitol, fructose, galactose, mannose, rhamnose, arabinose, xylose, sucrose, lactose, maltose and partially raffinose. No ability to utilize inositol and sorbitol was detected. Strain SK7 utilized acetate, fructose, galactose, glucose, mannitol, mannose, ribose, xylose, lactose, and raffinose as carbon sources.

Determination of streptomycetes heavy metal resistance. Among the studied metals, chromium and iron were the least toxic for all strains; while zinc and cobalt

exhibited moderate toxicity toward the studied strains (**Fig. 3**). Notably, that treatment with these increased levels of cobalt and chromium induced the formation of a brown pigment in strain 89, which is likely a targeted response to stress, suggesting that the synthesis of this pigment may be an essential component of the strain's resistance mechanisms. Nickel and copper proved to be the most toxic: growth inhibition of strain SK7 was observed at 25 mg/L of nickel, strain 34 at 200 mg/L, while strain 89 was able to grow at 300 mg/L. Copper at 50 mg/L inhibited growth of 34 and SK7; only strain 89 tolerated 100 mg/L of copper and up to 900 mg/L of chromium. All strains grew in the presence of iron up to 700 mg/L.

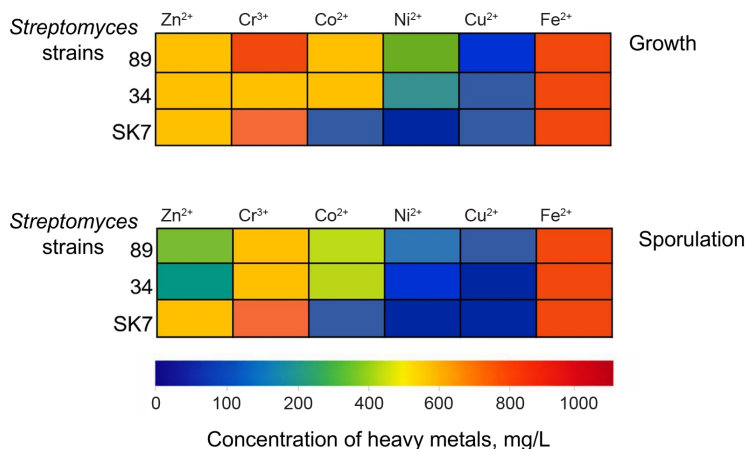


Fig. 3. Maximum tolerated concentrations of heavy metals that support the growth and sporulation of *Streptomyces* strains. Heatmaps represent the tolerance thresholds (mg/L) of strains 89, 34, and SK7 to various metal cations on PGA medium

The presence of heavy metals in the nutrient medium suppressed sporulation more strongly than growth. For example, *Streptomyces* sp. SK7 grew at 50 mg/L, but sporulation was already inhibited at 25 mg/L of copper. Notably, *S. anulatus* 89 demonstrated significantly higher tolerance to nickel compared with SK7 (300 mg/L compared with 25 mg/L) and twice the tolerance to copper compared with both 34 and SK7, which highlights its significantly higher physiological resistance to heavy metals.

The analysis of streptomycetes antagonistic activity. The largest inhibition zones of the tested fungi were observed on PGA, and smaller ones on Chapek–Doks agar (**Table 2**). Among the *Streptomyces* strains, 89 inhibited *B. sorokiana*, *F. oxysporum* and *A. arborenses*. The antifungal activity of strains SK7 and 34 was low, neither 34 nor 89 inhibited the tested phytopathogenic bacteria or opportunistic microorganisms. In contrast, strain SK7 weakly inhibited *S. aureus* ATCC 25923 (4.0 ± 0.18 mm). Non-parametric statistical analysis confirmed that *S. anulatus* 89 exhibited significantly larger zones of inhibition against major phytopathogens compared with strains 34 and SK7. For example, its antagonistic activity against *B. sorokiana* on PDA medium (up to 13.7 ± 0.47 mm) was approximately three times higher than that of the other isolates. This effect was highly significant (Kruskal–Wallis $H = 14.65$, exact $p = 0.00065$, $\epsilon^2 = 0.73$), indicating a remarkably large effect size. Subsequent pairwise comparisons using Dunn's post-hoc test with Bonferroni correction confirmed that the activity of strain 89 was significantly higher than that of strain 34 (adjusted $p = 0.0249$) and SK7 (adjusted

$p = 0.0006$). Furthermore, its activity against *B. sorokiana*, *F. solani*, *A. arborensense*, and *F. culmorum* did not significantly differ in comparison with the effect of nystatin. Based on this statistically robust superior bioactivity, combined with notably higher tolerance to toxic metals (nickel and copper) and the broader enzymatic profile, strain 89 was selected as the primary and the most promising candidate for further genomic characterization.

Table 2. Antifungal properties of *Streptomyces* strains, $\pm$ SD, $n = 3$

Fungi	Streptomyces strains					Nystatin 80 U (positive control)
	89, NM 1*	89, NM 2	89, NM 3	34, NM 2	SK7, NM 2	
	Inhibition zones (mm)					
1	10.5 ± 0.08 ^a	13.7 ± 0.47 ^a	12.3 ± 0.47 ^a	3.33 ± 1.25 ^b	2.67 ± 0.47 ^b	15.0 ± 0.05 ^a
2	3.7 ± 0.47 ^a	4.3 ± 0.94 ^a	0 ^b	1.33 ± 0.47 ^b	4.0 ± 0.42 ^a	4.3 ± 0.47 ^a
3	9.0 ± 0.82 ^a	12.3 ± 1.25 ^a	11.7 ± 0.94 ^a	2.67 ± 0.47 ^b	2.67 ± 0.47 ^b	5.3 ± 0.47 ^{ab}
4	10.0 ± 0.09 ^a	11.3 ± 0.94 ^a	9.3 ± 0.471 ^a	4.0 ± 0.10 ^b	4.0 ± 0.01 ^b	7.7 ± 0.47 ^a
5	1.1 ± 0.01 ^b	1.0 ± 0.01 ^b	1.0 ± 0.01 ^b	1.0 ± 0.01 ^b	3.33 ± 0.47 ^a	8.7 ± 4.16 ^a
6	4.0 ± 0.82 ^a	6.7 ± 0.47 ^a	5.0 ± 0.09 ^a	2.67 ± 0.47 ^b	2.33 ± 0.47 ^b	6.7 ± 0.58 ^a
7	5.0 ± 0.82 ^b	5.0 ± 0.09 ^b	3.3 ± 0.94 ^c	4.67 ± 0.53 ^b	4.0 ± 0.01 ^b	7.7 ± 0.58 ^a
8	1.7 ± 0.47 ^b	2.7 ± 0.47 ^b	1.0 ± 0.09 ^c	2.0 ± 0.47 ^b	1.33 ± 0.47 ^c	6.7 ± 0.58 ^a
9	1.3 ± 0.94 ^b	0 ^c	0 ^c	1.0 ± 0.94 ^b	1.68 ± 0.47 ^b	3.0 ± 0.06 ^a

Notes: NM – nutrient medium, 1 – PGA; 2 – PDA modified; 3 – Czapek–Dox agar with glucose. Fungi: 1 – *B. sorokiana* TR1; 2 – *F. solani* ATCC 11712; 3 – *F. oxysporum* TR1; 4 – *A. arborensense* TR1; 5 – *F. oxysporum* DSM 841; 6 – *F. culmorum* DSM 21713; 7 – *F. graminearum* DSM 21727; 8 – *R. solani* DSM 22880; 9 – *A. niger* ATCC 6275. Different letters within a column indicate statistically significant differences according to the Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction ($P \leq 0.05$).

Whole-genome analysis of strain 89. Previous whole-genome sequencing of *S. anulatus* 89 (BioProject PRJNA1215024) enabled a high-resolution phylogenetic analysis of the strain. The average nucleotide identity (ANI) to the reference genome of *S. anulatus* JCM 4721 was 97.48 %, which significantly exceeds the generally accepted threshold for species differentiation (95–96 %), thereby definitively confirming the taxonomic affiliation of strain 89 to *S. anulatus* (Sylchuk *et al.*, 2026). The genome-encoded resistance mechanisms associated with osmotic and oxidative stress, carbon starvation, periplasmic stress, and detoxification pathways were detected. Thus, encoded proteins may contribute to protection against salinity, namely aquaporin Z, glycerol uptake facilitator, propanediol diffusion facilitator, outer membrane protein A, OsmY. Proteins encoded for heavy metal resistance included mercuric reductase, cobalt-zinc-cadmium resistance, and copper tolerance and homeostasis. Remarkably, the presence of an encapsulating protein for DyP-type peroxidase and ferritin-like protein oligomers was detected, conferring tolerance to high concentrations of metals in the environment. Several proteins associated with antibiotic resistance have been identified, including topoisomerase IV (subunits A and B), DNA gyrase (subunits A and B), and the efflux pump Lde contributing the antibiotic resistance (Brettin *et al.*, 2015). CARD analysis enabled the identification of resistance genes for 39 classes of compounds, with the largest number of genes associated with resistance to macrolides, tetracyclines, and fluoroquinolones.

Analysis of the *S. anulatus* 89 genome using antiSMASH identified a total of 36 putative BGCs, indicating the strain's high biosynthetic potential. To study the identified antifungal activity of this strain, we focused on three specific BGCs that show homology with known clusters of antifungal compounds (**Fig. 4**).

Region 3.1 was identified as a type III polyketide synthase (T3PKS) cluster, showing homology with the naringenin BGC from *S. clavuligerus* ATCC 27064. Region 5.2, a RiPP-type cluster, showed high similarity to the streptamidine cluster from *S. albidoflavus* J1074. Furthermore, region 11.1, a mixed cluster of type II PKS and oligosaccharides, demonstrated significant structural conservatism and high identity to the warkmycin CS1 BGC from *Streptomyces* sp. CS057. The presence of these structurally conserved regions suggests that strain 89 has high biotechnological potential.

Antibiotic susceptibility test. The antibiotic susceptibility profile of strain 89 is presented in **Table 3**. The largest inhibition zones were found for doxycycline, amikacin, and kanamycin.

Table 3. The antibiotic susceptibility profile of *S. anulatus* 89, ($\pm$ SD, n = 3)

Antibiotics, μ g		Growth retardation zones, mm	Less dense lawn, mm	Color loss zones, mm
Polyenes	Nystatin, 80 U	0	0	0
	Amphotericin, 40	0	0	0
Glycopeptides	Teicoplanin, 30	0	2.00 ± 0.01	0
	Vancomycin, 30	5.67 ± 0.47	0	0
Amino-glycosides	Gentamicin, 120	8.00 ± 0.02	0	0
	Kanamycin, 30	9.33 ± 0.89	0	0
	Streptomycin, 10	7.00 ± 0.02	0	0
	Amikacin, 30	10.01 ± 0.8	0	0
	Ampicillin / Sulbactam, 10/10	0	0	0
Penicillins	Amoxicillin, 30	0	2.00 ± 0.01	0
	Amoxicillin, 10	0	0	0
	Ampicillin, 10	0	0	0
	Penicillin G, 6 U	0	0	0
Tetracyclines	Tetracycline, 30	0	3.00 ± 0.8	14.00 ± 1.63
	Ofloxacin, 6	0	0	0
	Doxycycline, 30	13.01 ± 0.82	0	20.01 ± 0.82
Fluoro-quinolones	Levofloxacin, 5	0	3.33 ± 0.47	11.3 ± 1.70
	Ciprofloxacin, 5	0	2.00 ± 0.01	13.33 ± 1.2
Macrolides	Erythromycin, 15	0	1.00 ± 0.01	0
	Azithromycin, 15	0	1.00 ± 0.02	0
	Clarithromycin, 15	0	2.00 ± 0.01	0
β-Lactams	Cefazolin, 30	0	0	0
	Amoxicillin / Clavulanic acid, 20/10	0	3.00 ± 0.01	0
	Ceftriaxone, μ g	0	0	0
	Imipenem-cilastatin, 10/10	2.67 ± 0.47	0	9.00 ± 2.16
Polymyxins	Polymyxin B, 300 U	0	0	0

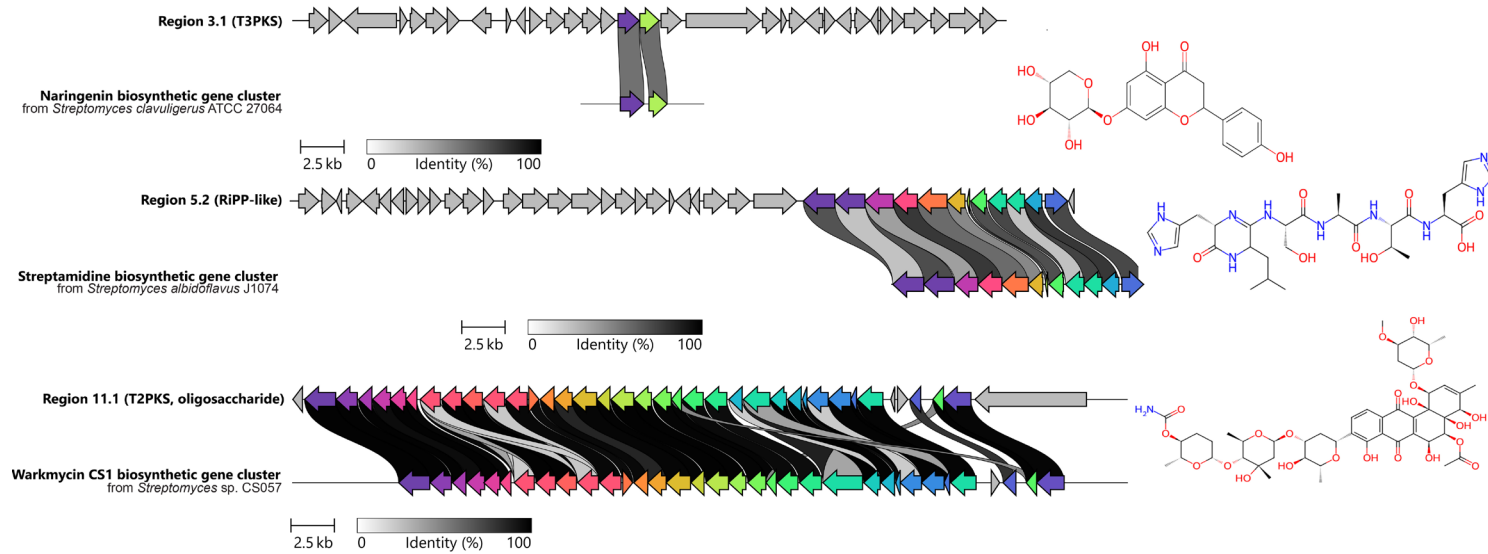


Fig. 4. Gene cluster comparison of the selected BGCs from *S. anulatus* 89. The alignment, generated using Clinker v0.0.32, visualizes the sequence identity and spatial organization of the regions 3.1 (T3PKS), 5.2 (RiPP-like), and 11.1 (T2PKS) against the known BGCs for naringenin, streptamidine, and warkmycin CS1, respectively. Greyscale links between genes indicate the percentage of amino acid identity. The illustrations of the chemical structures of the compounds were adapted from The Natural Products Atlas (Poynton *et al.*, 2024)

No inhibition zones were observed under the action of polyenes, teicoplanin, 30 µg, ampicillin/sulbactam, 10/10 µg, tetracycline, 30 µg; ofloxacin, 6 µg, penicillins, fluoroquinolones, macrolides, cefazolin, 30 µg; amoxicillin/clavulanic acid, 20/10 µg; ceftriaxone, 30 µg, and polymyxin B, 300 U. The genome-encoded resistance to macrolides, tetracyclines and fluoroquinolones resistance was confirmed by the test. Fluoroquinolones and macrolides reduced the lawn density of the streptomycete. Notably, tetracyclines and fluoroquinolones induced pigment loss (Fig. 5).

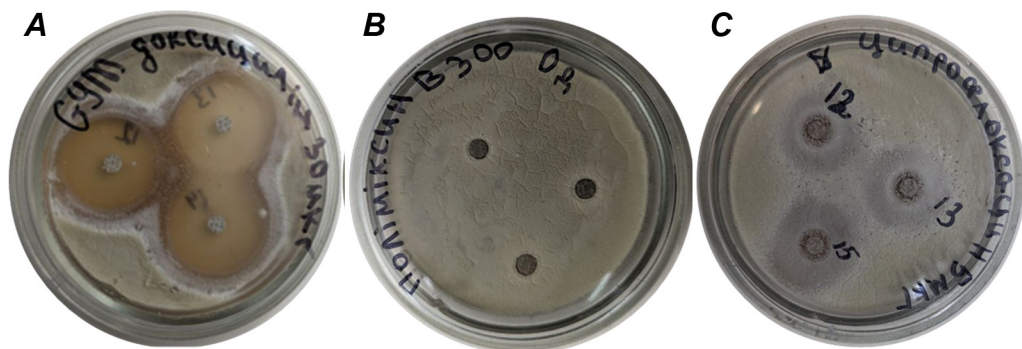


Fig. 5. Antibiotic susceptibility profile of *S. anulatus* 89 on GYM medium. The panels illustrate: (A) strong growth inhibition by doxycycline (30 µg); (B) lack of inhibition zones, demonstrating resistance to polymyxin B (300 U); and (C) stress-induced loss of pigmentation and reduced lawn density around ciprofloxacin (5 µg) disks

DISCUSSION

The studied strains exhibited minor antifungal activity but broad adaptive potential. Among them, *S. anulatus* 89 was selected as possessing promising biotechnological potential, which can be further enhanced by optimizing cultivation conditions to express antifungal activity. The obtained antifungal properties of *S. anulatus* 89 may be linked to the identification of BGCs showing high homology to known bioactive compounds, such as warkmycins, streptamidine and naringenin. All these compounds are likely fungicidal, in particular naringenin may inhibit the growth and development of phytopathogenic fungi by binding to glucokinase (Guo *et al.*, 2025), and its ability to stimulate plant resistance may be utilized in crop production (Sun *et al.*, 2022). A cluster highly homologous to the streptamidine cluster, a peptide, containing a structurally rare amidine ring, has also been identified (Russell *et al.*, 2021). Such compounds are regarded as promising bioactive agents, including fungicides (Zainal Abidin *et al.*, 2024). Furthermore, a cluster homologous to the warkmycin synthesis cluster was identified; warkmycins are compounds with high cytotoxicity and potential antifungal properties (Helaly *et al.*, 2013). Obviously, it cannot be excluded that the antimicrobial effect of strain 89 may be associated with other compounds, as its genome contains many clusters with low homology. GYM medium was identified as optimal for maximum biomass accumulation. This is confirmed by common practice, as organic ingredients are more economical, contain amino acids, vitamins and trace elements, and better maintain a stable pH level (Loboda *et al.*, 2021).

Assessment of carbon source utilization revealed that the studied strains metabolize a broad spectrum of carbon sources. Such versatility not only strengthens resilience

to environmental factors but also facilitates the optimization of nutrient medium composition for subsequent strain cultivation in biotechnological applications, adapted to the availability of resources and different cultivation goals, such as rapid biomass growth and increased production of antibiotics (Krysenko & Wohlleben, 2024). The ability of *S. anulatus* 89 to hydrolyze casein, together with the activity of lipase, amylase and cellulase, emphasizes its potential role in the decomposition of organic residues in soil.

One of the most common environmental challenges faced by microorganisms in their natural habitats is the fluctuation of external osmotic conditions. Soil-dwelling streptomycetes, in particular, frequently encounter sharp shifts triggered by rainfall or drought and have developed sophisticated mechanisms to cope with changes in osmolality (Bhowmick *et al.*, 2023). The studied strains 89, 34, and SK7 grew in nutrient media containing elevated NaCl concentrations. This indicates broad ecological plasticity, even when not originating from saline biotopes, as in the case of SK7. Therefore, they can be assigned to the group of moderate halophiles, with SK7 demonstrating adaptive flexibility despite its isolation from non-saline soil. This ability highlights their potential ecological versatility and biotechnological relevance. As revealed by sequencing results, the genome of *S. anulatus* 89 contains genes that may contribute to the development of cellular osmotolerance. Such resilience includes the genome-encoded proteins (aquaporin Z, glycerol uptake facilitator, propanediol diffusion facilitator, outer membrane protein A, OsmY) (Bremer & Krämer, 2019). Together, these proteins may potentially provide a coordinated mechanism that supports the resilience. Additionally, osmotolerance may be employed in the bioremediation of saline or degraded soils, where conventional microorganisms fail to survive. Moreover, salt-tolerant streptomycetes remain capable of producing antibiotics, enzymes, and other secondary metabolites under osmotic stress, which is advantageous for industrial fermentation processes (Hashim *et al.*, 2026).

S. anulatus 89 grew and sporulated under elevated concentrations of heavy metals, including Zn^{2+} , Cr^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Fe^{2+} , with copper proving to be the most toxic. The least toxic metal for the studied isolates was iron, which corresponds to the general trend for streptomycetes (Djinni & Djoudi, 2022). The resistance of strain 89 may be linked with the presence of genome-encoded determinants. This includes RND family proteins (resistance, nodulation and cell division), which are part of transmembrane complexes that detoxify the periplasm by exporting toxic metal cations to the extracellular space. As reported, they usually act together with cytoplasmic efflux systems, thereby creating a protective mechanism against transition metals (Bisht *et al.*, 2026). The identified laccase activity of *S. anulatus* 89 may potentially contribute to this resistance, as they oxidize toxic compounds, reduce oxidative stress, and complement genetic resistance determinants in *Streptomyces* spp., making laccase-producing strains valuable for biotechnology (Jiang *et al.*, 2025). For example, research on *Streptomyces* in the tomato rhizosphere showed that they can activate selenium and passivate cadmium, thereby reducing metal toxicity and supporting plant growth (Zulfiqar *et al.*, 2023). An unusual halotolerant and alkaline laccase from *S. psammoticus* was described, which was activated by metal ions such as Fe, Zn, Cu, Na, and Mg (Niladevi *et al.*, 2008). The encoded BGC of melanin may also contribute to the resistance due to the ability of this molecule to chelate ions of heavy metals and inhibit the Fenton reaction (Tran-Ly *et al.*, 2020, Adebajo *et al.*, 2023).

S. anulatus 89 was resistant to many classes of antibiotics, which is characteristic for soil streptomycetes, in particular because they are producers of a wide range of them and have developed appropriate defence mechanisms in the process of evolution (Thomy *et al.*, 2025, Nguyen *et al.*, 2025). For example, the encoded topoisomerase IV and DNA gyrase are essential enzymes that maintain DNA topology and supercoiling, and they serve as primary targets for fluoroquinolone antibiotics, while the efflux pump Lde actively expels antimicrobial compounds from the cell. The presence of these resistance determinants potentially contributes to the resistance and underpins the ability of strain 89 to survive in adverse environments (Silva *et al.*, 2023). It should be noted that a single plasmid was previously identified in its genome; however, it does not carry antibiotic resistance genes. Instead, it is associated with resilience under nutrient-limited soil conditions and confers a competitive advantage by supporting robust growth, sporulation, and antibiotic biosynthesis (Sylchuk *et al.*, 2026). Therefore, stress-prone habitats, including war-impacted or saline soils, represent valuable sources for isolating streptomycetes with broad adaptive potential and promising biotechnological applications. Among the studied strains, *S. anulatus* 89 was identified as the most promising for further investigation, based on its antagonistic activity, heavy metal resistance, and previously described biosynthetic potential (Sylchuk *et al.*, 2026).

LIMITATIONS AND FUTURE EXPERIMENTAL VALIDATION

Despite the promising findings, several limitations constrain the application of *S. anulatus* 89. First, the observed antifungal activity was moderate and requires further optimization. Second, while genome-encoded determinants and laccase activity suggest potential mechanisms of resistance, their functional roles, as well as osmotolerance, remain to be experimentally confirmed.

CONCLUSIONS

The studied *Streptomyces* strains, isolated from adverse biotopes, are characterized by ecological plasticity and exhibit resistance to saline and heavy metal ions. Although in the present study the strains demonstrated moderate antimicrobial activity, they possess comparatively wider adaptive potential and are promising for further investigations aimed at enhancing antagonistic properties. Particular attention should be given to strain 89, which previously demonstrated anticancer properties together with confirmed encoded BGCs (Sylchuk *et al.*, 2026). In addition to these antibiotics, its genome encodes BGCs for 14-hydroxyisochainin, warkmycin, and kinamycin, suggesting that it may potentially display additional antimicrobial activities.

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AUTHOR CONTRIBUTION

Conceptualization, [M.L.]; methodology, [A.S.; M.L., I.R.; T.R.]; validation, [M.L.; G.I.]; formal analysis, [M.L.; G.I.; I.R.]; investigation, [M.I.; G.I.]; resources, [M.L.; T.R.]; data curation, [M.L.]; writing – original draft preparation, [A.S.; M.L.; I.R.; G.I.; T.R.]; writing – review and editing, [A.S.; M.L.; I.R.; G.I.]; visualization, [A.S.; M.L.] supervision, [M.L.]; project administration, [T.R.]; funding acquisition, [T.R.].

Data availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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АДАПТИВНИЙ І БІОТЕХНОЛОГІЧНИЙ ПОТЕНЦІАЛ ШТАМІВ *STREPTOMYCES*, ВИДІЛЕНИХ ІЗ НЕСПРИЯТЛИВИХ БІОТОПІВ

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Обґрунтування. Представники *Streptomyces*, виділені з несприятливих біотопів, характеризуються широкими адаптаційними можливостями, унікальним метаболізмом і біотехнологічним потенціалом. У цьому дослідженні штами, ізольовані з несприятливих середовищ, проаналізовано за допомогою філогенетичного аналізу, біотестів і оцінки їхнього адаптаційного та біотехнологічного потенціалу.

Матеріали та методи. Використано генетичні методи (виділення ДНК та секвенування генів 16S rRNA), мікробіологічні методи (метод агарових блоків для вивчення антагонізму; культивування на живильних середовищах із додаванням важких металів для визначення металорезистентності; диско-дифузійний метод Кірбі–Байєра для тестування антибіотикочутливості), біохімічні методи (тести на ензиматичну активність і метаболізм джерел карбону), а також біоінформатичні інструменти (молекулярна філогенія в MEGA12.1.1; анотація геному в RAST і DFAST).

Результати. Філогенетичний аналіз показав близьку спорідненість між штамми 89, 34 та SK7, які утворили одну кладу філогенетичного дерева. Вони виявляли помірну протигрибну активність. Серед них штам 89 інгібував гриби родів

Fusarium і *Rhizoctonia*. Хром і залізо виявилися найменш токсичними для всіх штамів, цинк і кобальт – помірно токсичними, тоді як нікель і мідь – найбільш токсичними. Пригнічення росту відбувалося за концентрації 25 мг/л нікелю та 50 мг/л міді; лише штам 89 витримував 100 мг/л міді. Штам 89 виявляв ліпазну, амілазну та целюлазну активність, а також здатність гідролізувати казеїн і метаболізувати гліцерин, маніт і різні цукри, що підтверджує його потенціал у деградації органічних залишків.

Висновки. *S. anulatus* 89 має широкий адаптаційний і біотехнологічний потенціал та інгібує деякі фітопатогени.

Ключові слова: *Streptomyces*, несприятливі біотопи, антагонізм, фітопатогени, важкі метали, резистентність, ензиматична активність