https://doi.org/10.30970/apmicro.2026.43

Loboda M., Sylchuk A.

STREPTOMYCES SPP. FROM WAR-IMPACTED SOILS: WHOLE-GENOME ANALYSIS, METABOLITE PROFILING, AND ANTAGONISTIC ACTIVITY AGAINST PHYTOPATHOGENS

Department of General and Soil Microbiology, D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine,
154 Akademika Zabolotnoho St., Kyiv, 03143, Ukraine
e-mail: mariialoboda@imv.org.ua

Abstract. Fifty streptomycete isolates were recovered from war-impacted soils, of which nine of the most antagonistically active strains were selected for further study based on dual-culture screening against fungi and bacteria. Strains Y15, SK4, SK14 and SK16 displayed the strongest and broadest inhibitory activity, with Y15 producing the largest inhibition zones overall, notably against *Bipolaris sorokiniana* (27.9 ± 0.2 mm) and *Xanthomonas campestris* (15.8 ± 0.5 mm). Whole-genome sequencing and orthoANI analysis identified *Streptomyces* sp. SK4 as *S. lidicus* (100 % identity), while Y15, SK16 and SK14 showed at most 88–92 % identity to their closest relatives. Genome mining with antiSMASH revealed a conserved biosynthetic core shared by Y15 and SK4 alongside seven strain-specific biosynthetic gene clusters in each, and LC-HRMS profiling confirmed production of reedsmycin F and youssoufene A1 by Y15 and streptolydigin by SK4. These findings highlight war-impacted soils as a valuable, underexplored source of biotechnologically promising streptomycetes.

Keywords: *Streptomyces,* war-impacted soil, whole-genome sequencing, biosynthetic gene clusters, antagonistic activity, antibiotics.

Streptomyces spp. are well known as a prolific biofactory of secondary metabolites (Alam et al., 2022). The isolation of novel strains from unfavourable environments, including war-impacted soils, is a valuable source of bioactive representatives, since such strains may combine the adaptive potential required for survival under stress with a unique, biotechnologically valuable metabolism. The aim of this study was to screen *Streptomyces* spp. isolated from war-impacted soils, perform whole-genome sequencing, identification and genome annotation, determine their antifungal and antibacterial activity, and carry out metabolite profiling of the most active strains.

Streptomycete strains were cultivated on GYM medium without prior chemical treatment. Whole-genome sequencing (Illumina NovaSeq) was performed at Explogen LLC (Lviv, Ukraine); Average Nucleotide Identity (ANI) was calculated using the FastANI algorithm implemented in DFAST_QC (Elmanzalawi et al., 2025), and biosynthetic gene clusters (BGCs) were identified using antiSMASH 8.0 (Blin et al., 2025). Antifungal activity was assessed by a modified dual-culture confrontation method, and antibacterial activity by lawn seeding of test bacteria followed by agar-block application and measurement of the inhibition zone. Metabolite profiling of the most promising strains was performed by LC-HRMS (Dionex Ultimate 3000 RSLC coupled to a Bruker maXis 4G hr-ToF mass spectrometer).

In total, 50 isolates were recovered, of which 9 were selected for further study based on their antagonistic activity; strains Y15, SK4, SK14 and SK16 proved most promising. Y15 strain exhibited the largest inhibition zones overall, notably against *Bipolaris*

sorokiniana TR2 (27.9 ± 0.2 mm), *Alternaria arborescens* TR1 (17.5 ± 0.6 mm) and *Fusarium oxysporum* DSM 21731 (15.8 ± 1.7 mm), and the highest antibacterial activity, against *Xanthomonas campestris* IMV 80036 (15.8 ± 0.5 mm). SK16 showed the strongest bacterial suppression after Y15 strain (*Agrobacterium tumefaciens* IMV 8628 and *X. campestris*, 11.8 ± 0.5 mm each) with moderate-to-strong fungal inhibition (up to 11.3 ± 1.2 mm). SK4 train produced large zones against both fungal (*A. arborescens* TR1, 15.7 ± 0.6 mm) and bacterial (*X. campestris* IMV 80036, 10.6 ± 1.3 mm) agents, while SK14 generated the single largest zone against *F. graminearum* DSM 21727 (16.0 ± 0.0 mm), though its antibacterial effect remained low.

By orthoANI analysis, Y15 strain showed the highest similarity to *S. youssoufiensis* JCM 18307 (91.51 %), while SK4 showed 100 % identity with *S. lidicus* ATCC 25470; SK16 was most similar to *S. aurantiacus* JCM 4677 (88.16 %) and SK14 to *S. coriariae* CMB-FB (89.35 %), indicating that Y15, SK16 and SK14 may potentially represent novel species. Genome mining with antiSMASH 8.0 revealed a conserved core of three shared BGCs in Y15 strain and *S. lidicus* SK4 – for isorenieratene, ectoine, and a piericidin-related T1PKS – alongside seven strain-specific clusters in each. *Streptomyces* sp. Y15 was enriched in arylpolyene/polyketide clusters (youssoufene A1 and analogues B1–B4, reedsmycins, echosides A–E, the diisonitrile antibiotics SF2768, nigericin, naseseazine C/C3-arylperroindolines, geosmin), whereas *S. lidicus* SK4 carried predominantly peptide- and siderophore-associated clusters (streptolydigin, the lanthipeptide SapB, the lassopeptide citrulassin D, antipain, desferrioxamine E, a spore-pigment T2PKS, and mannopeptimycin).

LC/MS analysis confirmed that *Streptomyces* sp. Y15 produces reedsmycin F ($[M+H]^+$ m/z 667.4013, RT 7.1 min), a non-glycosylated polyene that binds fungal ergosterol and disrupts membrane integrity, and youssoufene A1 ($[M+H]^+$ m/z 537.2969, RT 14.2 min), a cinnamoyl lipid antibiotic active against Gram-positive bacteria such as *S. aureus*, while *S. lidicus* SK4 produces streptolydigin ($[M+H]^+$ m/z 601.3094, RT 10.8 min), an RNA-polymerase-targeting antibiotic active against *S. aureus* and *B. subtilis*. These results experimentally validate the BGCs predicted by antiSMASH genome mining.

Thus, war-impacted soils proved to be a valuable, underexplored reservoir of biotechnologically promising streptomycetes. Strain Y15 combines the broadest antagonistic spectrum with biosynthetic capacity for both antifungal (reedsmycin F) and antibacterial (youssoufene A1) compounds, making it the most promising candidate for biotechnologies. *S. lidicus* SK4 is a source of the antibiotic streptolydigin, of interest for agricultural and biomedical use, while SK16 and SK14 are additional candidates for targeted biocontrol of bacterial and *Fusarium*-associated phytopathogens, respectively.

References

1. Alam, K., Mazumder, A., Sikdar, S., Zhao, Y.-M., Hao, J., Song, C., Wang, Y., Sarkar, R., Islam, S., Zhang, Y., & Li, A. (2022). *Streptomyces*: The biofactory of secondary metabolites. Frontiers in Microbiology, 13, 968053. https://doi.org/10.3389/fmicb.2022.968053
2. Blin, K., Shaw, S., Vader, L., Szenei, J., Reitz, Z. L., Augustijn, H. E., Cediel-Becerra, J. D. D., de Crécy-Lagard, V., Koetsier, R. A., Williams, S. E., Cruz-Morales, P., Wongwas, S., Segurado Luchsinger, A. E., Biermann, F., Korenskaia, A., Zdouc, M. M., Meijer, D., Terlouw, B. R., van der Hooft, J. J. J., ... Weber, T. (2025). antiSMASH 8.0: Extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation. Nucleic Acids Research, 53(W1), W32–W38. https://doi.org/10.1093/nar/gkaf334

3. Elmanzalawi, M., Fujisawa, T., Mori, H., Nakamura, Y., & Tanizawa, Y. (2025). DFAST_QC: Quality assessment and taxonomic identification tool for prokaryotic genomes. BMC Bioinformatics, 26(1), 3. https://doi.org/10.1186/s12859-024-06030-y