

## ASSESSMENT OF ANTIBIOTIC RESISTANCE IN A GRAPEVINE-DERIVED *Bacillus* sp. ISOLATE

Tatjana Dujković<sup>1</sup>, Ivana Danilov<sup>1</sup>, Vanja Vlajkov<sup>1</sup>, Jovana Grahovac<sup>1</sup>

Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi  
Sad, Serbia

e-mail: ivana.pajcin@uns.ac.rs

### Abstract

The irresponsible and excessive use of antibiotics has contributed to the rapid spread of antimicrobial resistance (AMR), which has emerged as a major global health concern. In this context, special attention must be devoted to the use of microorganisms in various sectors in order to minimize the risk of horizontal transfer and dissemination of AMR genes. In the present study, *Bacillus* sp. 17/S was isolated from the grapevine stem and investigated for its potential as a biocontrol agent. As an essential first step, the antimicrobial resistance profile of the isolate was evaluated. An *in vitro* antibiotic susceptibility assay was conducted using the disk diffusion method, and results were interpreted based on inhibition zone diameters: isolates were considered susceptible ( $\geq 21$  mm), moderately susceptible (16–20 mm), or resistant ( $\leq 15$  mm). In addition, the presence of antibiotic resistance genes was analyzed by the PCR (polymerase chain reaction)-based method. Several genes associated with different resistance mechanisms including *penP*, *blaOXA*, *vmiR*, *lmrB*, *ant-6*, *aac-3*, *tetL*, and *mphK* were selected as the molecular targets. The antibiotic susceptibility tests revealed the highest level of resistance towards  $\beta$ -lactam antibiotics, with the isolate being resistant to oxacillin, aztreonam, methicillin, cefepime, and cefixime, while showing moderate susceptibility to ceftaroline ( $20.00 \pm 0.00$  mm) and piperacillin ( $19.67 \pm 0.58$  mm). In contrast, the susceptibility was observed to penicillin G ( $37.33 \pm 0.58$  mm), ampicillin ( $22.33 \pm 0.58$  mm), imipenem ( $43.67 \pm 0.58$  mm), amoxicillin ( $29.00 \pm 1.00$  mm), and cephalixin ( $23.67 \pm 0.58$  mm). Within the lincosamide group, the isolate was susceptible to clindamycin ( $21.67 \pm 0.58$  mm) and moderately susceptible to lincomycin ( $15.00 \pm 0.00$  mm). Among tetracyclines, susceptibility was observed to minocycline ( $24.67 \pm 0.58$  mm), with moderate susceptibility to tetracycline ( $17.00 \pm 0.00$  mm) and doxycycline ( $18.67 \pm 1.53$  mm). Regarding aminoglycosides, the isolate was susceptible to gentamicin ( $24.67 \pm 0.58$  mm) and streptomycin ( $24.67 \pm 0.58$  mm), while tobramycin ( $19.00 \pm 0.00$  mm), kanamycin ( $19.00 \pm 0.00$  mm), and amikacin ( $18.67 \pm 2.08$  mm) exhibited only moderate activity, and the resistance was observed to neomycin. All tested macrolides (azithromycin, erythromycin, and clarithromycin) displayed strong growth inhibitory effects. Notably, none of the investigated resistance genes corresponding to these antibiotic groups was detected in the isolate's genomic DNA. It can be concluded that the *Bacillus* sp. 17/S isolate displays a heterogeneous antibiotic susceptibility profile. The absence of the tested resistance genes suggests that phenotypic resistance may not always be explained by the known genetic determinants. These findings highlight the importance of comprehensive safety evaluations of microbial strains prior to their potential application as biocontrol agents.

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