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PCR SCREENING AND CHEMICAL ANALYSIS OF LIPOPEPTIDES PRODUCED BY BACILLUS VALEZENSIS AND BACILLUS MEGATERIUM STRAINS

Aleksandra Jelušić¹, Tatjana Popović², Ivica Dimkić³, Petar Mitrović⁴, Slaviša Stanković³, Sanja Marković¹, Tanja Berić³

1 Institute for Multidisciplinary Research, Belgrade, Serbia

2 Institute for Plant Protection and Environment, Belgrade, Serbia

3 Faculty of Biology University of Belgrade, Belgrade, Serbia

4 Institute of Field and Vegetable Crops, Novi Sad, Serbia

Species belonging to the genus *Bacillus* are known for their ability to suppress various plant pathogenic fungi and bacteria, due to synthesis of secondary metabolites like antibiotics and lipopeptides. The main lipopeptide families responsible for the antimicrobial activity of *Bacillus* species are fengycins, iturins, kurstakins and surfactins.

Objectives:

The aim of this study was to identify and test two *Bacillus*-like isolates which showed in vitro antagonistic potential against phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* for the presence of lipopeptide genes.

Methods:

For identification, DNA of tested isolates was amplified with primers based on 16S rRNA and *tuf* genes and sequenced. The presence of lipopeptide genes was detected using primer pairs ITUP1-F/ITUP1-R, AKS-F/TKS-R, FEND1-F/FEND1-R, BACC1-F/BACC1 and P17/P18 for detection of the genes for iturin, kurstakin, fengycin and bacillomycin D synthetases and surfactin, respectively. The obtained PCR products were checked for correct size and sequenced. The liquid chromatography-mass spectrometry (LC-MS) was used to confirm presence of the searched lipopeptides.

Results:

Based on the obtained sequences for 16S rRNA and *tuf* gene, isolates were identified as *Bacillus valezensis* and *Bacillus megaterium*. The results of PCR screening showed that both strains have genes for biosynthesis of kurstakines and surfactines, based on the obtained band of correct size (1125–1175 bp and 675 bp, respectively). *B. valezensis* strain showed presence of the gene for bacillomycin D synthetase, producing the band on the position of 800 bp. Presence of these molecules was also confirmed with the LC-MS.

Keywords: lipopeptides, antagonism, *Bacillus*, *Pseudomonas*