ICGEB Meeting and Courses 2023

ICGEB WORKSHOP

TRENDS IN MICROBIAL SOLUTIONS FOR SUSTAINABLE AGRICULTURE

13 – 15 September 2023. Belgrade, SERBIA

BOOK OF ABSTRACTS

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Trends in microbial solutions for sustainable agriculture

13 – 15 September 2023. Belgrade, Serbia

Edition

Collection of the Faculty of Biology

ISSN 3009-3740 (Online)

Publisher

University of Belgrade, Faculty of Biology
Studentski Trg 16, 11158 Belgrade, Serbia
www.bio.bg.ac.rs

For publisher

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Technical Editor & Cover design

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Internation Standard Book Number

ISBN 978-86-7078-178-8

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Outer membrane vesicles of plant beneficial bacterial strain *Paraburkholderia phytofirmans* PsJN make a contact with *Arabidopsis thaliana* roots

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Extracellular vesicles (EVs) are recognized as important mediators of intercellular communication in both eukaryotes and prokaryotes. These lipid membrane – coated spherical nanoparticles carry proteins, nucleic acids and other cellular products, and facilitate exchange of these biomolecules among cells within an organism, but also between cells of different organisms, belonging to different species and even kingdoms. Outer membrane vesicles (OMVs), EVs produced by Gram-negative bacteria, are a significant mediator of microbial communication, involved in biofilm formation, virulence, and modulation of host immunity. OMVs of both pathogenic and plant beneficial bacteria have been shown to elicit plant immune responses. Investigations on the modes of OMV-plant cells interactions are still in their infancy, but gain rising attention. Aiming to monitor the interaction between OMVs of *Paraburkholderia phytofirmans* PsJN, a plant growth promoting bacteria, and *Arabidopsis thaliana* roots, we isolated OMVs from bacterial culture in mineral medium, using an ion-exchange chromatography system. Isolated OMVs were labeled with lipid binding fluorescent dye Vybrant™ DiD and unbound dye was removed by washing vesicles on ultrafiltration columns. The same dye concentration in phosphate buffer saline, equivalently washed, was used as a control. *A. thaliana* roots, grown on Murashige and Skoog medium, were incubated with DiD-OMVs or control dye/buffer mixture, washed and observed under confocal laser scanning microscope. Red signals were observed in root hairs and epidermis in DiD-OMV treated plants, while in control-treated roots the same signals were missing. The results indicate direct contact of bacterial vesicles with epidermis and root hairs, which are indispensable for nutrient acquisition and plant-microbe interactions in rhizosphere. Further investigation will address the questions of the nature of OMV-plant cell interaction, including potential delivery of OMVs cargo into host plant cells. Considering that OMVs are increasingly recognized as promising tools in biomedicine, exploring their potential for agronomical applications would be highly appreciated.

Keywords: outer membrane vesicles (omvs); *Paraburkholderia phytofirmans* PsJN; intercellular communication.