Assessment of the effect of outer membrane vesicles of endophytic bacteria on the growth and physiological response of *Arabidopsis thaliana*

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MOTIVATION

The world faces the challenge to produce a sufficient amount of food for the constantly growing human population in a sustainable manner. It means the usage of pesticides and fertilizers with minimized adverse effects on the environment and wide application of the beneficial microorganisms. Bacteria produce outer membrane vesicles (OMVs) that might affect plant development.

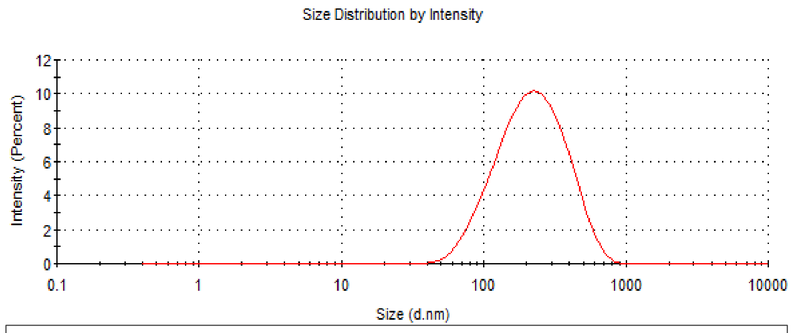
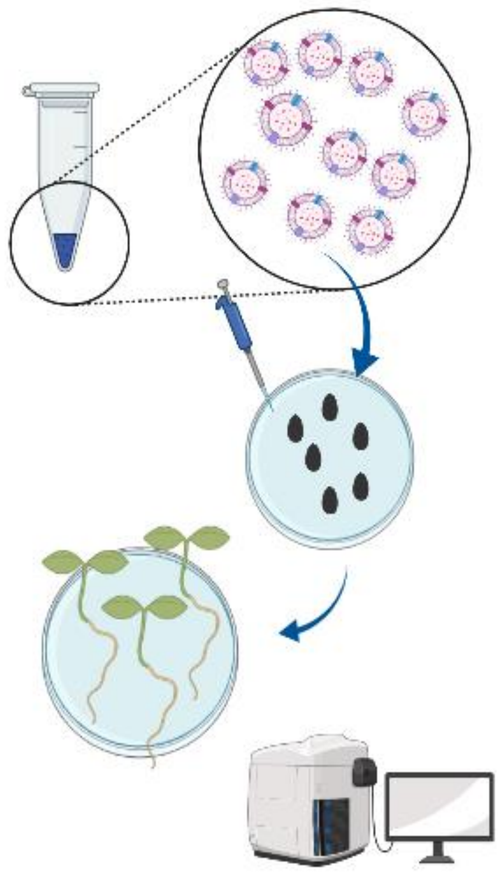


Fig. 1. The OMV *Serratia* sp. diameter were analyzed using the DLS intensity-weighed distribution (Zetasizer, Malvern).



RESULTS

- Isolation of OMVs from endophytic bacteria strictly depends on the strain and conditions of growth. The crucial role in this process seemed to be connected with the bacterial species.
- All bacterial strains produced OMVs with sizes *Serratia* sp. 180.4±1.71 nm; *Pseudomonas* sp. L1 163.55±4.45 nm; *P. agglomerans* 2CJKA 110.5±2.39 nm (Fig. 1)
- Experiments conducted so far have shown that OMVs produced by tested strains had a great ability to induce germination, especially the OMV from *Pseudomonas* sp. L1. (Fig. 2A).
- The OMVs originated from the tested bacteria differently influenced the length of plant roots. OMV *Pseudomonas* sp. L1 promoted root growth.
- *Serratia* sp. OMVs inhibited the growth of *A. thaliana* seedlings whereas OMVs released by *Pseudomonas* sp. stimulated it (Fig. 2B).
- Furthermore, OMVs produced by both bacterial strains had a great effect on the activity of plant oxidative enzymes (Fig. 2C). The MDA concentration was the lowest when OMV *Pseudomonas* sp. L1 was added to the Arabidopsis seeds.
- The highest MDA concentration was observed in Arabidopsis seedlings after OMV from *Serratia* sp. treatment.

CONCLUSION

The knowledge of plant response to OMVs is limited. Therefore, the preliminary studies seem to be important to obtain knowledge that may be applicable in the development of the new natural compounds used as plant growth stimulators.

The OMVs production by endophytic strains is connected with different plant seedlings' reactions. Promote root growth and reduce the time of germination indicate the positive influence of OMVs on plant growth and development. Especially when we add to the analysis plant oxidative enzymes. The tendency to stress reduction by endophytic bacterial OMVs seems to be one of the applications to reduce the negative effect of pathogens and/or environmental conditions on plant fitness.

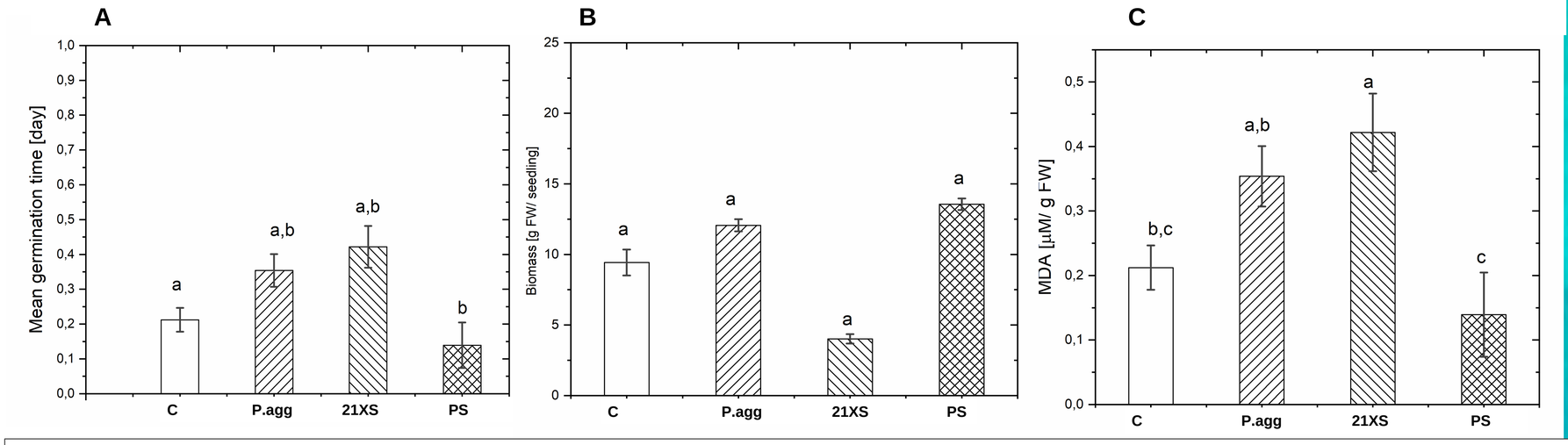
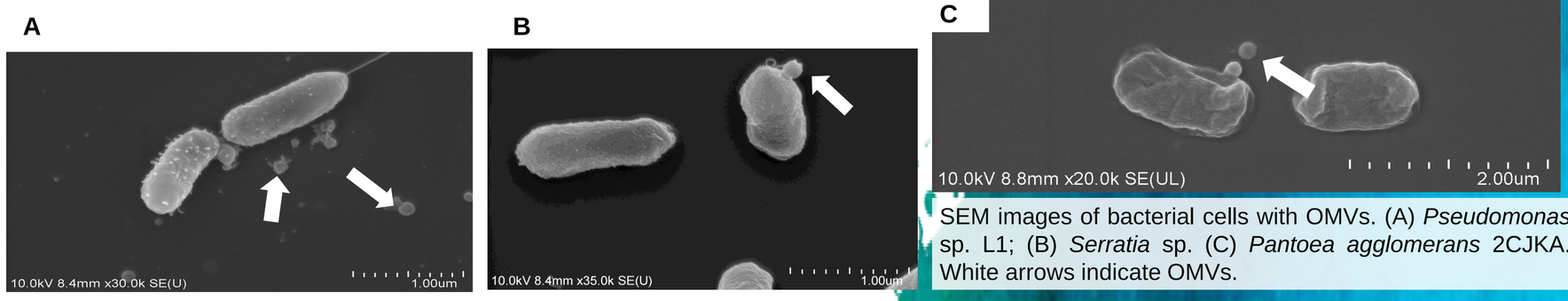


Fig. 2. The OMV influence on *Arabidopsis thaliana*. (A) germination, (B) fresh biomass of seedlings, (C) MDA concentration. Presented are the means and standard deviations (SD). Means followed by different letters (a, b, c) are significantly different (LSD test $p < 0.05$). Abbreviations: C–control, P. agg–OMV *Pantoea agglomerans* 2CJKA; 21XS–OMV *Serratia* sp.; PS–OMV *Pseudomonas* sp. L1.



SEM images of bacterial cells with OMVs. (A) *Pseudomonas* sp. L1; (B) *Serratia* sp. (C) *Pantoea agglomerans* 2CJKA. White arrows indicate OMVs.

AIM and EXPERINMENTAL

The aim of the study was to evaluate the impact of OMVs on the *Arabidopsis thaliana* condition. The experiments were conducted with OMV produced by endophytic bacteria.

To obtain OMVs the specific protocols for their isolation have been developed. Bacterial strains *Pseudomonas* sp. L1, *Serratia* sp. and *Pantoea agglomerans* 2CJKA were cultured in 2-6 L of LB liquid medium (28°C, 120 rpm) until the late logarithmic phase. The bacterial culture was centrifugated (9000 rpm, 20 min.). The supernatant was passed through filters (0.45 and 0.22 µm) and then transferred to an ultrafiltration tube with a molecular weight cutoff of 100 kDa under a final volume 100-200 ml. The concentrated liquid was ultracentrifuged to pelleted the OMVs. The crude OMV was then subjected to OptiPrep density gradient (10-45%) and ultracentrifuged. The purified OMV accumulated at the 20-40% interface were collected and resuspended in PBS. The ultracentrifugation was carried out using Beckman 45 Ti fixed angle rotor 30 000 rpm, 2-4 h, 4°C and/or 50.2 Ti fixed angle rotor 30 000 rpm, 18-24 h, 4°C (L-80 Optima Beckman). The protein concentration of the prepared OMVs was measured (BSA method). The OMVs morphology was performed with the use of SEM. OMVs were measured by Dynamic Light Scattering (DLS) using the Zetasizer Nano ZS (Malvern Instruments) to detect the size distribution.

OMVs were added on the surface of sterilized seeds of *A. thaliana* that were grown on Murashige and Skoog agar medium for 21 days. To assess the impact of OMVs on plants the rate of seeds germination, the biomass of shoots and roots were measured. Moreover, the level of oxidative stress and activity of antioxidant enzymes in plants were evaluated (MDA, malondialdehyde). MDA is a marker of lipid peroxidation.

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