Extracellular vesicles as communication tool in plant-bacteria interactions

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Introduction

Extracellular vesicles are nano-sized membrane structures, produced and secreted by prokaryotic and eukaryotic cells, that are mainly involved in cell-to-cell communication[1]. They contain and carry many molecules and macromolecules - such as cellular metabolites, proteins, nucleic acids (DNAs, sRNAs) - through which they could interact with target cells at molecular levels.

Scientific background from our laboratory

In our laboratory different actinobacteria strains have been assayed for their Plant Growth Promoting (PGP) traits[2]. In particular, a *Streptomyces violaceoruber* strain has been proven to possess different PGP traits such as indole acetic acid production, phosphate solubilization, N_2-fixation, drought and salt tolerance, bioactive molecules production, including antibiotics and siderophores. Different *in vivo* assays were performed on tomato (*Solanum lycopersicum L.*) and oregano (*Origanum vulgare L.*), resulted in an improved germination index and the growth of seedlings from seeds treated with PGP actinobacteria, with a particular focus on *S. violaceoruber* cultures, able to promote plant development through various processes (N_2-fixation, phosphate solubilization, auxin production, etc.).
A research regarding the treatment of grapevines buds with a culture of *S. violaceoruber* in Derxia medium[2], has shown how this plant-bacteria interaction is able to anticipate the exit from dormancy (manuscript in progress).

Aim of the work

The aim of the project is to isolate membrane vesicles (MVs) from *S. violaceoruber* strain, characterize them (through DLS analysis, SDS-page, agarose gel electrophoresis and genomic sequencing) and verify their effects on tomato seedlings in terms of plant growth promotion.

Isolation and Molecular Characterization

Isolation and characterization were performed through several analyses, that provide us many interesting informations at molecular level about MVs.

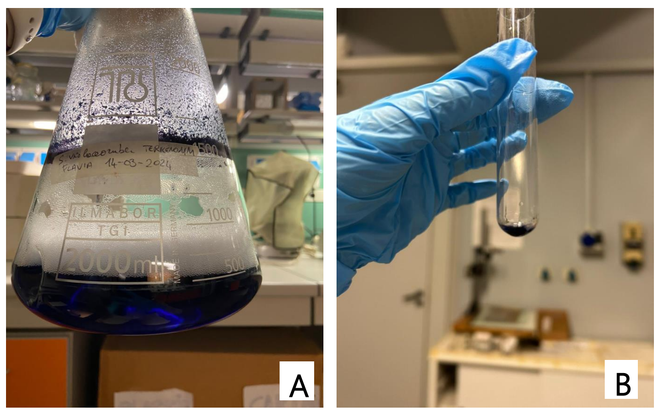

Figure 1: (A) Minimal medium colture of *S. violaceoruber* strain. **(B)** Pellet of MVs after ultracentrifugation (UC) step, according to the isolation protocol[3].

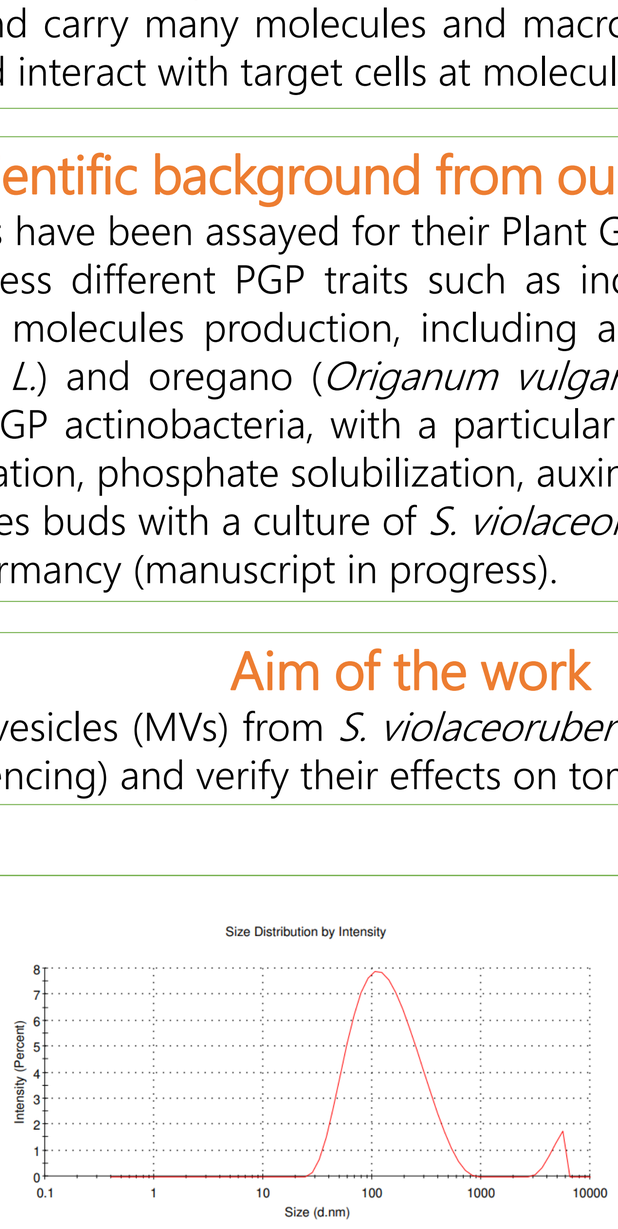

Figure 2: Size distribution profile of MVs in suspension through DLS analysis.

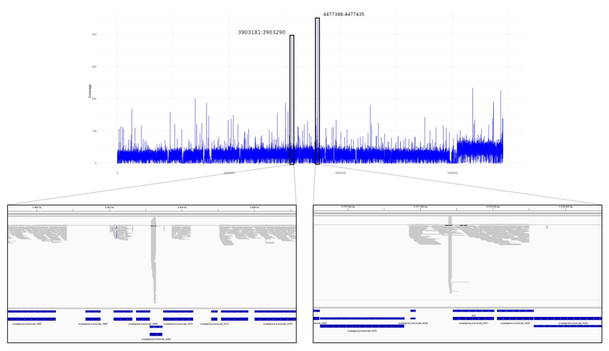

Figure 5: Mapping of sequencing reads of MVs on the reference chromosome sequence of *S. violaceoruber* comprising a zoom for specific regions spanning from 3903181 to 3903290 nt and from 4477388 to 4477435 nt.

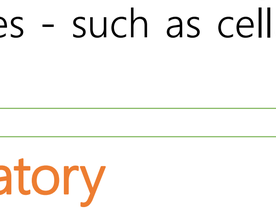

Figure 3: Proteic pattern of MVs through SDS-page. **(1)** SeeBlue Plus2 Prestained Standard. Proteic extract of MVs after a first **(2)** and second **(3)** UC step.

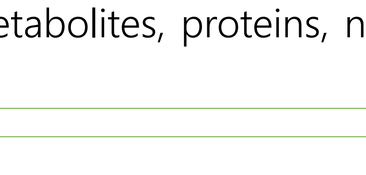

Figure 4: MVs nucleic acid content analysis by agarose gel electrophoresis. **(1)** MassRuler DNA Ladder Mix. Genomic extract of MVs after a first **(2)** and second **(3)** UC step.

Plant growth effects

In vivo tests were performed on tomato (*Solanum lycopersicum*) seedlings, treating the seeds with MVs suspension at various dilutions to analyze the effects on plant growth. An improvement of plant growth and development has been shown in the treated plants compared to the control ones (i.e. improved aerial and secondary root development, increased of root and shoot length).

Conclusion

S. violaceoruber MVs were isolated and characterized for their protein and nucleic acid content.
A Next Generetion Sequencing analysis approach revealed the presence of almost all the genomic DNA of the *S. violaceoruber* strain.
A stimulatory effect exerted by MVs was observed on tomato plant growth and development.
Further experiments will be performed to fully characterize the *S. violaceoruber* MVs cargo and to investigate other effects on plant growth and development.
These results suggest MVs as possible components to develop PGP-actinobacteria biofertilizers.

Figure 6: tomato (*S. lycopersicum*) seeds were sterilized and treated by soaking with a suspension of MVs at various dilutions in DPBS and arranged in squared plates (water and agar medium) for germination.

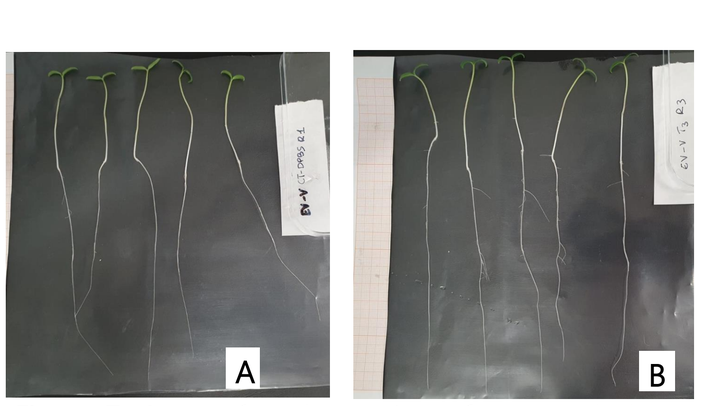

Figure 7: Set-up for observation of root and shoot differences in length and annotation of secondary root between control **(A)** and MVs treated plants **(B)** after 8 days from treatment.

Figure 8: Morphometric comparison between control (left) and MVs treated tomato plants (right) in peat discs and arranged in boxes with filter to allow gaseous exchanges. The latter shows a more developed epigeal apparatus compared to the control.

Acknowledgements

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References: **[1]** Urzì et al., 2021; Int.J.Mol.Sci.; 22,5366; **[2]** Faddetta et al., 2023; Metabolites; 13,374; **[3]** Faddetta et al., 2022; Scientific Reports; 12,16651.