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Extracellular Vesicles isolated from *Streptomyces violaceoruber*: an interesting natural tool for plant growth and health

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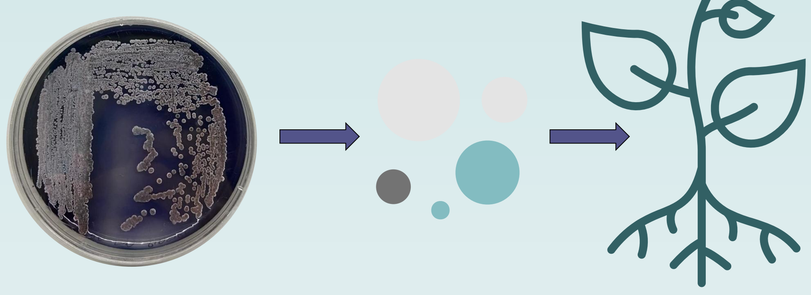
Introduction

Biofertilizers, based on Plant-Growth Promoting Bacteria (PGPB), can be used for a greener agriculture. Among these, *Streptomyces* species show significant potential due to their plant-growth promoting (PGP) traits. One key mechanism may involve extracellular vesicles (EVs), nanosized structures, carrying proteins, nucleic acids and bioactive compounds [1]. These vesicles are mainly involved in cell-to-cell communication and in the regulation of various trans-kingdom molecular mechanisms [2].



Aim and Methodology

The research focuses on the isolation, characterization of EVs from *Streptomyces violaceoruber,* a PGPB that is a prolific source of bioactive compounds [3]. They have been morphologically characterized and quantified through Scanning Transmission Electron Microscopy (STEM), Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA). Moreover, functional assays were performed, evaluating their antimicrobial and PGP capabilities on tomato plants (*Solanum lycopersicum*).

Results

Isolation, morphological characterization and quantification were performed through several analyses, which provide many interesting informations about the size distribution and concentration.

1. Isolation and Morphology

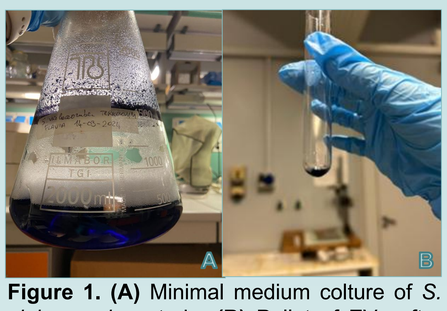


Figure 1. (A) Minimal medium colture of *S. violaceoruber* strain. **(B)** Pellet of EVs after isolation protocol, through ultracentrifugation step [4].

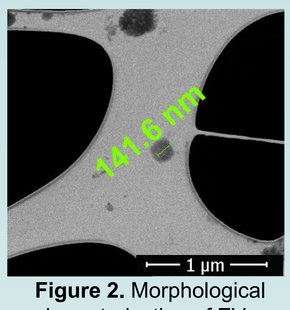


Figure 2. Morphological characterization of EVs through STEM analysis.

2. Quantification

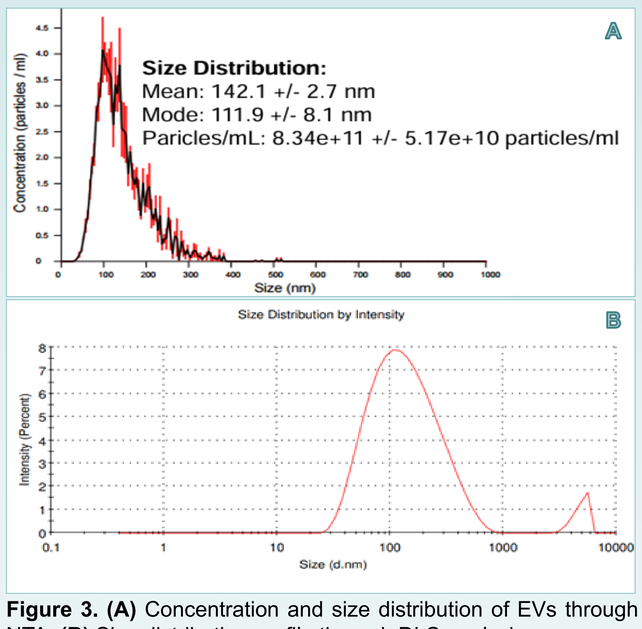


Figure 3. (A) Concentration and size distribution of EVs through NTA. **(B)** Size distribution profile through DLS analysis.

3. Genome sequencing

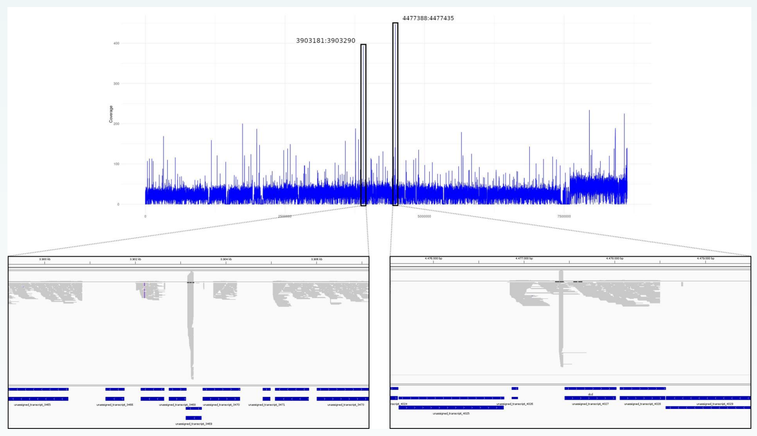


Figure 4. Mapping of DNA sequences of EVs, obtained by Next Generation Sequencing analysis, on the reference chromosome sequence of *S. violaceoruber.* Zoom of specific regions: the first one (spanning from 3903181 to 3903290 nt) falls into a pseudogene and the second one (from 4477388 to 4477435 nt) falls upstream of the gene encoding a dCTP deaminase.

Results

Preliminary evaluation of biological activities of EVs was performed investigating: ***i)*** antimicrobial activity; ***ii)*** PGP effects on tomato seedlings.

4. Antimicrobial activity

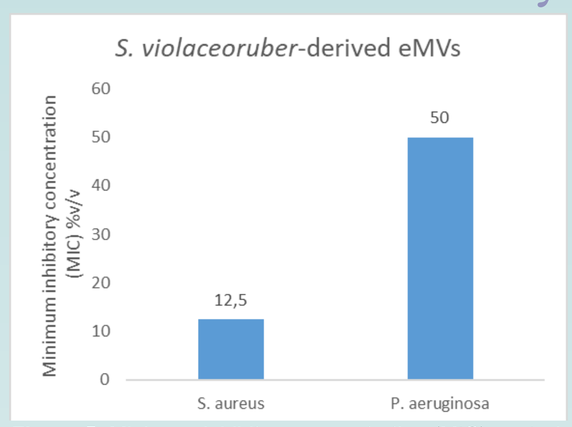


Figure 5. Minimum inhibitory concentration (MIC) analysis revealed the inhibitory growth activities of EVs against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at a 12.5% and 50% (v/v) respectively. Interestingly, EVs also showed bactericidal effect against *S. aureus*.

5. PGP effects

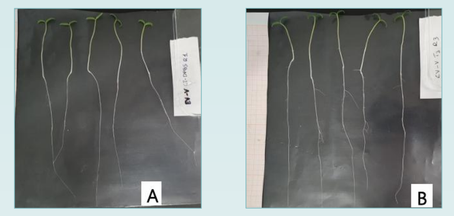


Figure 6. Observation of root differences in length and annotation of secondary root between control **(A)** and EVs treated plants **(B)** after 8 days from the treatment.

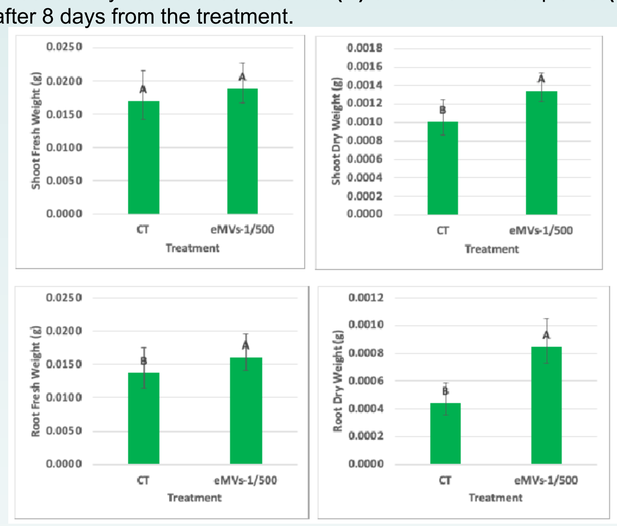


Figure 7. Weight measurements at 9 days of growth in tomato seedlings treated with EVs derived from *S. violaceoruber* at a 1/500 dilution of the stock solution, compared to untreated control seedlings. The treatment resulted in increased fresh and dry root weight, as well as increased shoot dry weight. Letters indicate statistically significant group differences ($p < 0.05$).

Conclusion

S. violaceoruber EVs - isolated for the first time and quantified - have been characterized for their morphological and molecular features. EVs showed some interesting biological effects, concerning the antimicrobial activity against human pathogens and PGP effects on tomato, revealing the EV potential role in trans-kingdom interactions. However, further investigations are needed to assess mode of action and molecular effects on EV-treated organisms.

Acknowledgements

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References

[1] Rutter et al., 2020; New Phytologist. 228:1505-1510; [2] Stotz et al., 2022; *FEMS Microbiology Reviews*, 1-18; [3] Faddetta et al., 2023; *Metabolites* 13,374; [4] Faddetta et al., 2022; *Appl. Environ. Microbiol*. 88, e01881-e1921.