

Extracellular Membrane Vesicles from *Streptomyces violaceoruber*: a promising natural strategy for enhancing plant growth and health

Flavia Cannizzaro¹, Teresa Faddetta^{1,2}, Claudia Bonafede¹, Noemi Sannasardo¹, Guglielmo Puccio³, Ciro Caldiero³, Loredana Abbate³, Valentina Ricciardi³, Sara Amata⁴, Carla Rizzo⁴, Giorgio Nasillo⁵, Sabrina Picciotto⁶, Antonio Palumbo Piccionello⁴, Antonella Bongiovanni⁶, Francesco Mercati³, Giuseppe Gallo^{1,2}

¹*University of Palermo, STEBICEF Department, Viale delle Scienze Ed. 16, 90128, Palermo, Italy.*

²*NBFC, National Biodiversity Future Center, Piazza Marina 61, 90133 Palermo, Italy.*

³*National Research Council, Institute of Biosciences and Bioresources (CNR-IBBR), Via Ugo La Malfa 153, Palermo, 90146, Italy.*

⁴*University of Palermo, STEBICEF Department, Viale delle Scienze Ed. 17, 90128, Palermo, Italy.*

⁵*Advanced Technologies Network (ATeN) Center Università degli Studi di Palermo Viale delle Scienze - Ed. 18/A, 90128, Palermo, Italy.*

⁶*National Research Council, Institute for Research and Biomedical Innovation (CNR-IRIB), Via Ugo La Malfa 153, Palermo, 90146, Italy*

Climate change and the growing demand for food necessitate eco-sustainable solutions to optimize resource use and reduce chemical inputs. In this context, biofertilizers, based on living Plant Growth Promoting (PGP) bacteria, offer a promising approach. Among PGP bacteria, *Streptomyces* species show significant potential. Streptomycetes are well known for their morpho-physiological differentiation and production of bioactive compounds. Extracellular membrane vesicles (eMV) - nanostructures naturally released by cells – can play key roles in intercellular communication, carrying proteins, nucleic acids, and other bioactive molecules. Therefore, this study focuses on the characterization of eMV isolated from the PGP bacterium *Streptomyces violaceoruber*, evaluating their morphology, cargo, and biological effects.

eMV were isolated through ultracentrifugation from *S. violaceoruber* six-day-old cultures grown in liquid minimal medium. Morphological, dimensional and quantitative characterizations were performed through scanning transmission electron microscopy, dynamic light scattering and nanoparticles tracking analysis. Molecular profiling was performed using omics-based approaches (*i.e.* next-generation sequencing, mass spectrometry analyses).

eMV, ranging from 100–200 nm in size, contain RNA and the whole *S. violaceoruber* genomic DNA. Additionally, they contain proteins, but their metabolic cargo has yet to be fully characterized. Interestingly, eMV exhibited antibacterial properties and showed PGP effects on tomato seedlings. Experiments are underway to evaluate their capability to mitigate plant biotic stresses, testing their effect against phytopathogens (*i.e.* *Verticillium* sp.) infections.

Therefore, this research aims to provide new evidence on the role of *Streptomyces*-derived eMV in trans-kingdom communication (microbe-microbe and plant-microbe interactions), and to lay the groundwork for the development of new eMV-based biofertilizers.