Biotechnological production of extracellular vesicles from *Coffea arabica* L. cell suspension cultures: isolation and proteomic profiling

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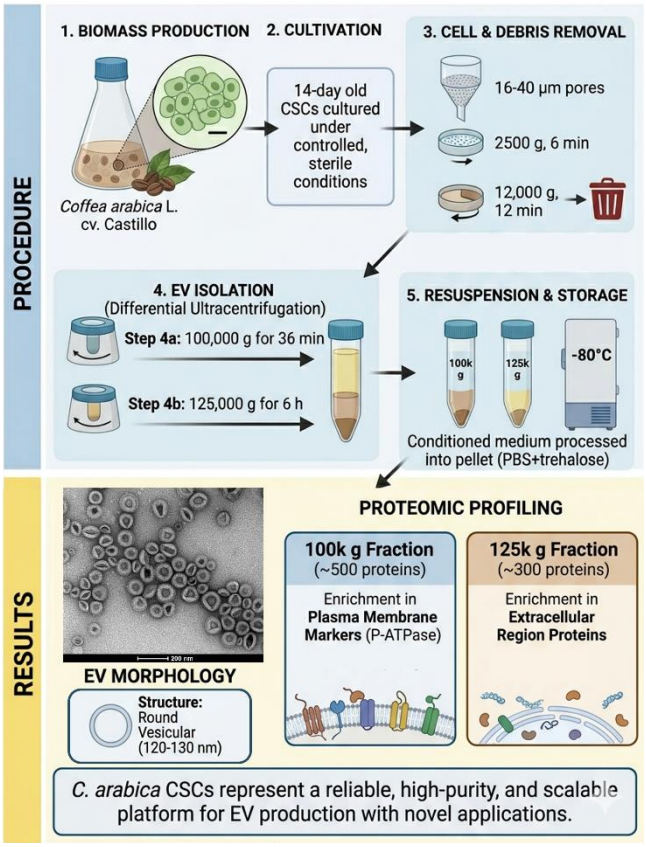
Extracellular vesicles (EVs) are emerging as a transformative category of biotherapeutics and biotechnological tools due to their pivotal role in intercellular communication and the transport of bioactive molecules, including proteins and nucleic acids. While EVs can be derived from several biological sources, plant-based systems offer significant advantages by minimizing risks related to immunogenicity and pathogen contamination. Specifically, plant cell suspension cultures (CSCs) provide a sterile, controlled, and scalable environment for high-purity EV recovery. In this study, we investigated the potential of *Coffea arabica* L. (cv. Castillo) CSCs as a reliable source of EVs. The vesicles were isolated from the conditioned culture medium of 14-day-old cells through a series of differential ultracentrifugation steps at 100,000 *g* (100k *g* for 36 min) and 125,000 *g* (100k *g* for 6 h). Morphological characterization via Transmission Electron Microscopy (TEM) confirmed the presence of round-shaped vesicles with an average diameter of approximately 120-130 nm. Proteomic profiling identified roughly 500 and 300 proteins in the 100k *g* and 125k *g* fractions, respectively. The 100k *g* fraction was primarily enriched with plasma membrane and cell periphery proteins, whereas the 125k *g* fraction contained proteins mostly associated with the extracellular region. Our findings demonstrate that coffee CSCs actively secrete EVs, likely through plasma membrane budding or unconventional secretory pathways. These results prove that *C. arabica* CSCs represent a highly efficient and scalable system for obtaining EVs without mechanical cell disruption. Such a platform opens new opportunities for downstream functional studies and innovative applications in the biotechnological and pharmaceutical sectors.



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References:

1. Wang & Kong, 2025 - doi: 10.1111/pbi.70074

2. Kırbaş et al., 2025 - doi: 10.1002/biof.2090