CHARACTERIZATION OF PROTEIN CARGO OF *Aeromonas hydrophila* EXTRACELLULAR VESICLES AND INTERACTIONS WITH HOST

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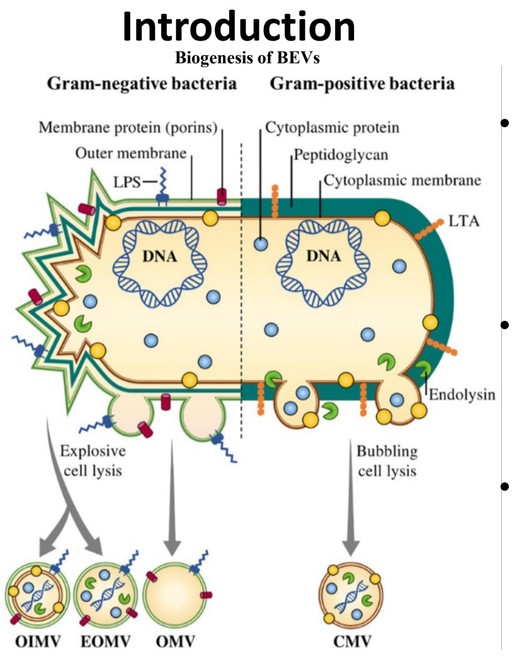
Abstract

Bacterial extracellular vesicles (BEVs) are recognized for containing diverse protein cargos including virulence factors and signaling molecules that promote cell communication and host-pathogen interactions. This study aims to characterize protein cargos of *Aeromonas hydrophila* BEVs (*Ah*EVs) and investigate their potential interactions with host through in silico analysis.

BEVs were isolated through ultracentrifugation and characterized. Proteomic analysis was conducted using an Extractive HF-X Hybrid Quadrupole-Orbitrap Mass Spectrometer along with the *A. hydrophila*-specific database. Identified proteins were subjected to subcellular localization and Gene Ontology (GO) annotation, followed by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. To elucidate potential biological functions between *Ah*EV proteins and the host, protein-protein interactions (PPIs) were predicted using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database.

TEM imaging confirmed that the ultrastructural morphology of the particles was spherical, and nanoparticle tracking analysis (NTA) determined their average size to be 105.5 ± 2.0 nm. Proteomic analysis identified 49,015 total spectra, 5,597 peptides (including 4,825 unique peptides), and 1,284 proteins. The most prevalent protein mass range was 20-30 kDa, comprising 228 proteins. Subcellular localization indicated that "cell" and "cell part" were the primary protein localization areas, together accounting for 46.32% of the structural proteins. GO analysis showed a higher number of proteins related to "catalytic activity" (566) compared to other functions. Biological processes were categorized into 19 functional annotations, with cellular process-related proteins being the most abundant (458). Protein-protein interactions (PPIs) with *A. hydrophila* proteins revealed 532 predicted interactions among the 1,284 identified *Ah*EV proteins. Additionally, KEGG pathway analysis identified 21 enriched pathways, related to various biological functions such as protein synthesis and transportation, RNA degradation as well as amino acid metabolism. Further PPIs with host (human) revealed 102 interactions where 88 pathway enrichments were observed from the KEGG analysis. Key pathways enriched were MAPK signaling, NF-κB signaling, Il17 signaling, toll-like receptor signaling, RIG-I-like receptor signaling, as well as NOD-like receptor signaling which are some of the crucial interconnected pathways regulating immunomodulatory effects. Further studies could unravel the functional role of specific protein cargo in *Ah*EVs.

Introduction

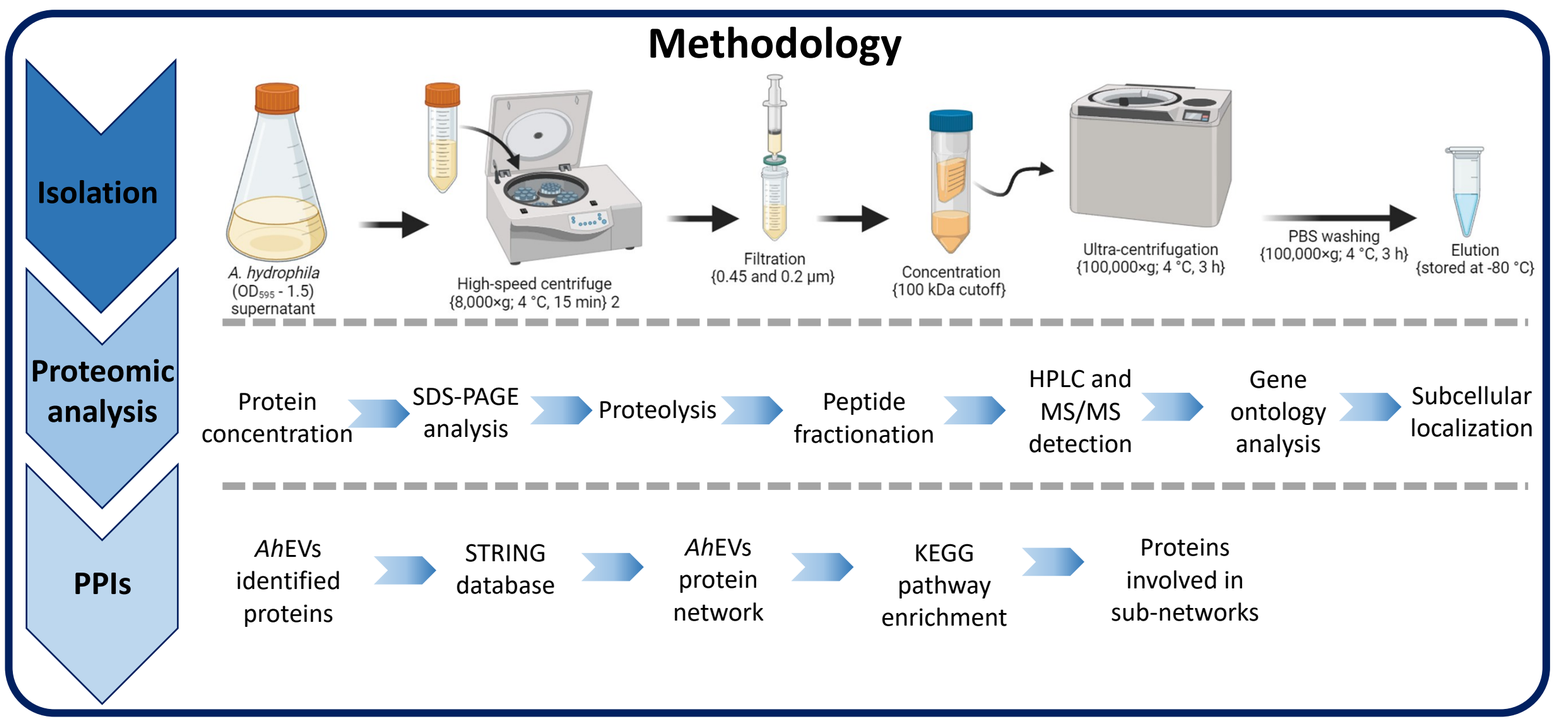


Source: https://doi.org/10.3389/fmicb.2021.785856

Objectives

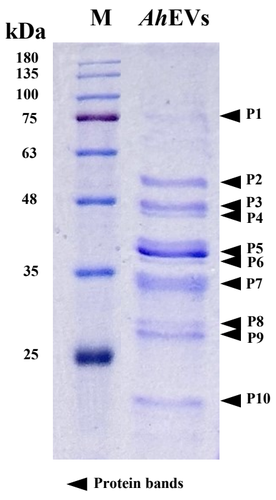
- Optimize the isolation procedure of *Ah*EVs using the ultracentrifugation method.
- Analyze the proteomic component of the isolated EVs.
- Evaluate the PPIs between the *Ah*EVs protein and host proteins.

Methodology



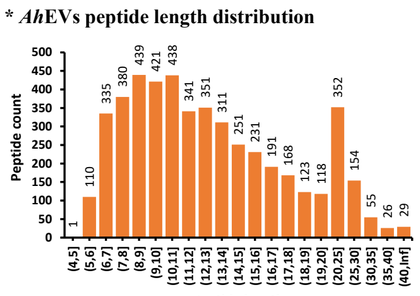
Results

* *Ah*EVs SDS-PAGE

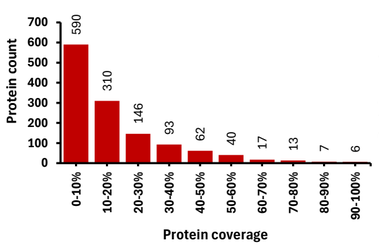


Total spectra	49,015
Identified spectra	9,515
Identified peptide	5,597
Identified (unique) peptide	4,825
Identified protein	1,284

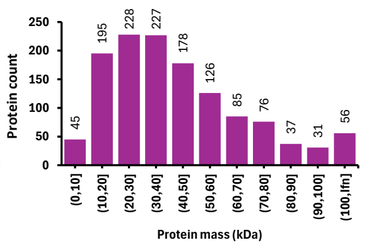
* *Ah*EVs peptide length distribution



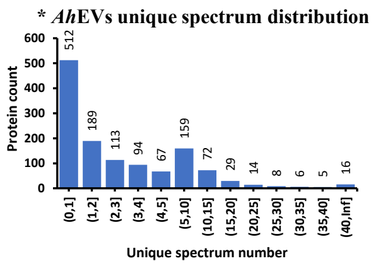
* *Ah*EVs protein coverage distribution



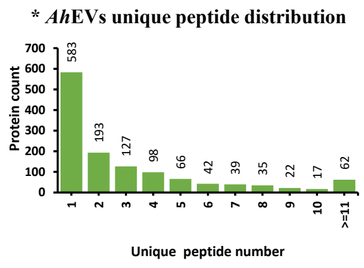
* *Ah*EVs protein mass distribution



* *Ah*EVs unique spectrum distribution



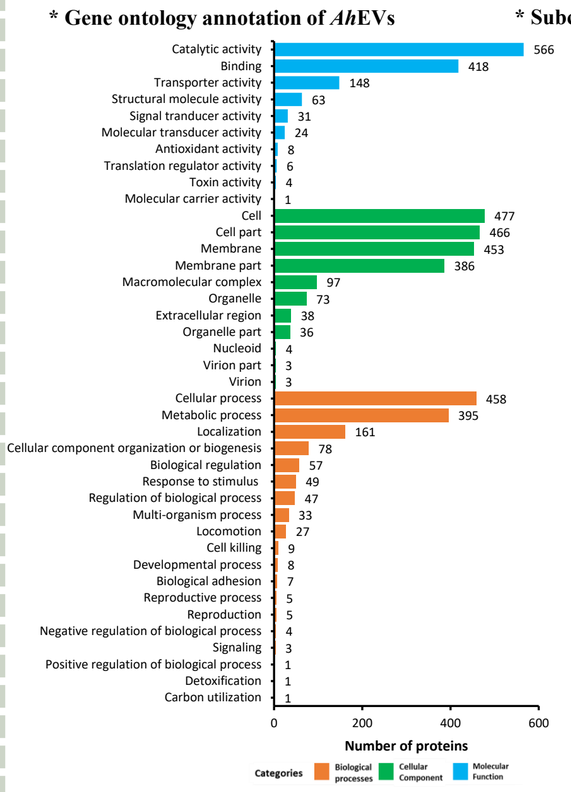
* *Ah*EVs unique peptide distribution



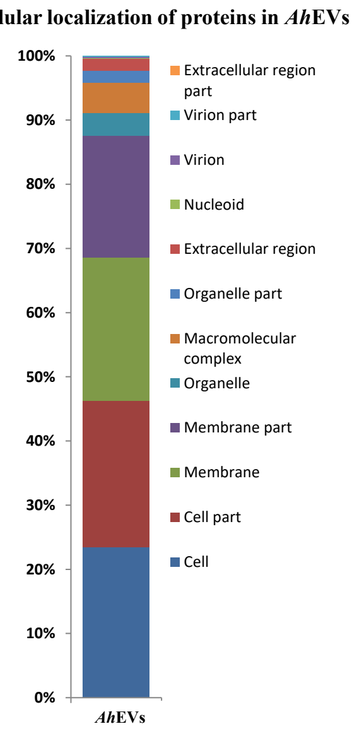
❖ The Coomassie-stained SDS-PAGE gel showed ten prominent protein bands for the *Ah*EV sample

❖ From the total spectra of 49,015, identified peptides of 5,597, and 4,825 were unique peptides

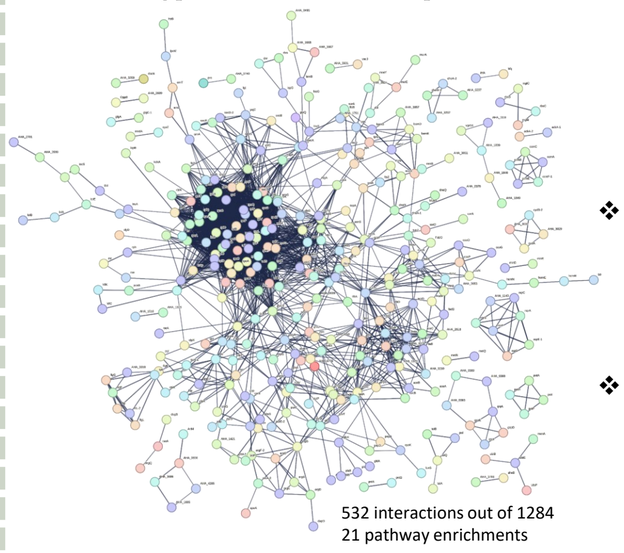
* Gene ontology annotation of *Ah*EVs



* Subcellular localization of proteins in *Ah*EVs



* Interacting protein network of *Ah*EVs proteins



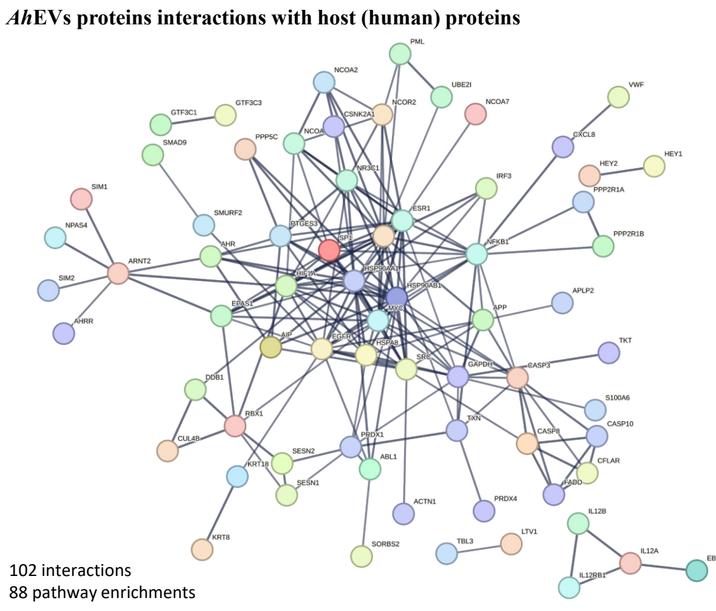
532 interactions out of 1284
21 pathway enrichments

❖ GO analysis showed 566 proteins involved in catalytic activity, while 458 proteins were known for cellular processes and 477 proteins involved for building up cell components

❖ Cell, cell part, membrane, and membrane part are the most abundant localized areas of the available proteins

❖ KEGG analysis showed 21 pathway enrichments from 532 interactions out of the 1284 *Ah*EVs proteins

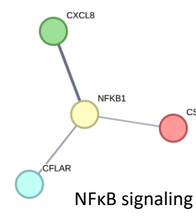
* *Ah*EVs proteins interactions with host (human) proteins



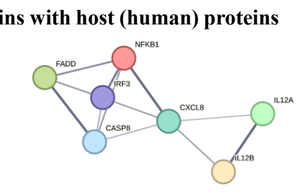
102 interactions
88 pathway enrichments

* Key interacting subnetworks of the *Ah*VEs proteins with host (human) proteins

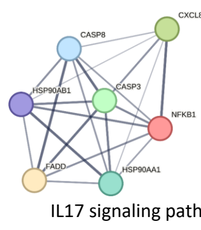
MAPK signaling pathway



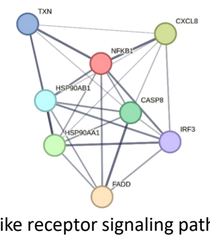
NFκB signaling



Toll-like receptor signaling pathway



IL17 signaling pathway



NOD-like receptor signaling pathway

❖ Out of the list, 14 *Ah*EVs proteins interacted with 117 host (human) proteins

❖ STRING database showed 102 interactions to make a network

❖ KEGG analysis showed 88 pathway enrichments

❖ Key sub-networks involved were related to immunomodulatory functions

Conclusions

❖ In the present study, isolation of EVs from the Gram-negative bacteria *A. hydrophila* was done using the ultracentrifugation method, resulting in ten protein bands, which were confirmed from SDS-PAGE analysis. Proteomic analysis revealed that *Ah*EVs consisted of 5,597 identified peptides, out of which 4,825 were unique peptides and 1,284 were identified proteins

❖ GO and subcellular localized protein analysis revealed that most of the proteins found in *Ah*EVs were cellular components belonging to cell, cell part, membrane, and membrane part

❖ PPIs of *Ah*EVs with *A. hydriphila* proteins revealed a network of 532 interactions and 21 pathway enrichments while when tested with host (human) proteins, it showed a network with 102 interactions. As some of the key sub-netwoks are heavily involved with immunomodulation further studies could reveal the potential use of such protein cargo when developing therapeutic drugs.

Reference: Kim, J.H., Lee, J., Park, J. and Gho, Y.S., 2015, April. Gram-negative and Gram-positive bacterial extracellular vesicles. In Seminars in cell & developmental biology (Vol. 40, pp. 97-104). Academic Press

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