The Role of OMVs in Plant Infection with *Xylella fastidiosa*

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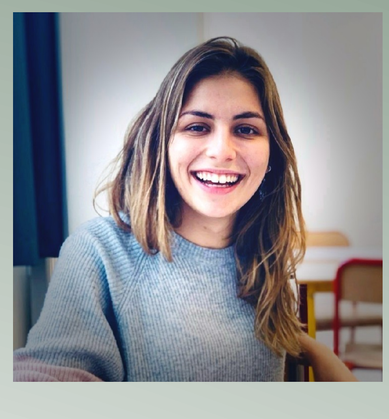


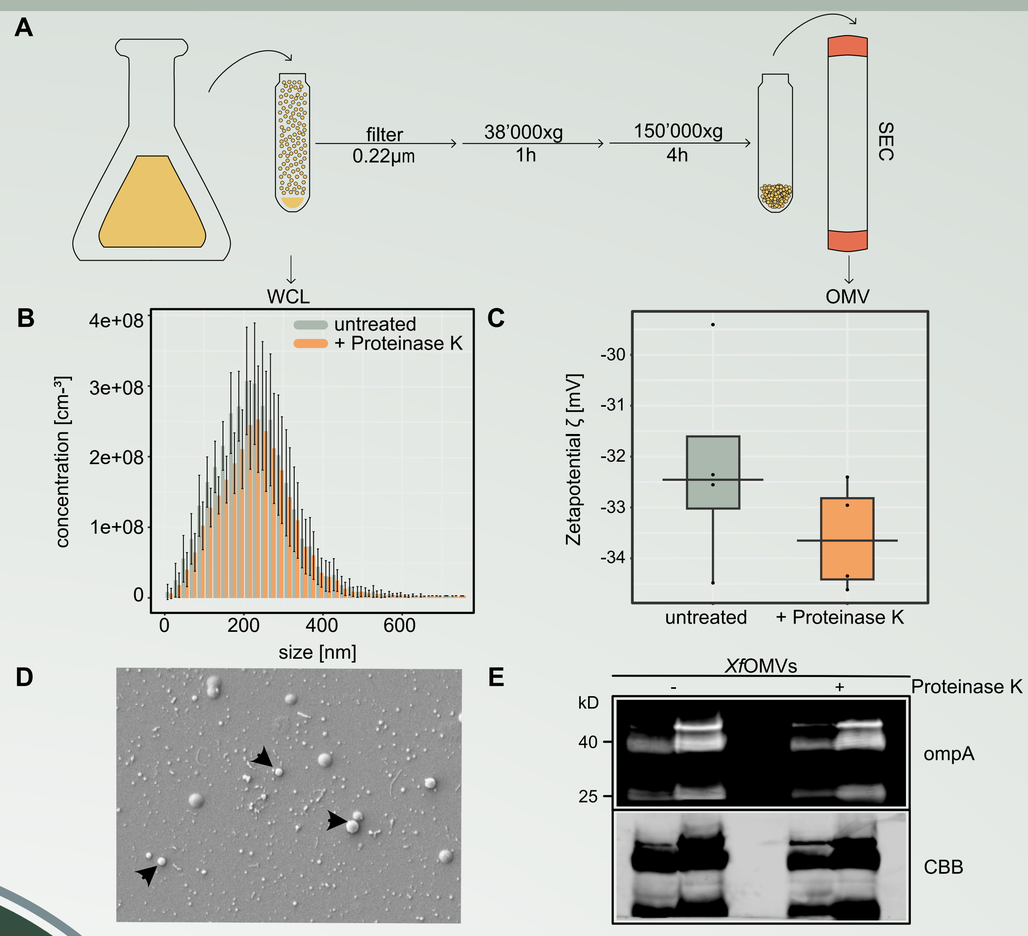
BACKGROUND

Xylella fastidiosa (*Xf*):

- gram-negative bacterial plant pathogen with many host species
- large part of virulence strategy not understood
- lacks type-III-secretion system to deliver effectors
- releases ca. 5x more outer-membrane vesicles (OMVs) than closely related species[1]
- little is known about cargo + role of OMVs for virulence

Our research aims to explore the composition of the external cargo (corona) and internal cargo of *Xf*OMVs in order to gain insights into their role in the bacterium's virulence and communication with surrounding cells. Our analysis has revealed proteins as *Xf*OMV cargo which are candidates for aiding infection. We further hypothesize that bacteria deliver RNA and RNA-binding proteins (RBPs) via OMVs to target cells. Understanding underlying mechanistics of *Xf*'s pathogenicity, could aid in developing tools against *Xf* infections, including antibacterial gene silencing.

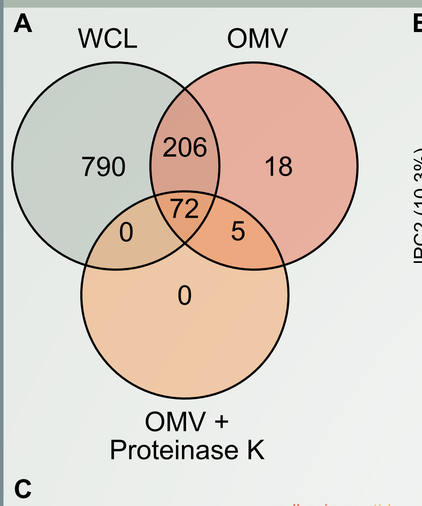
1. ISOLATION & CHARACTERIZATION

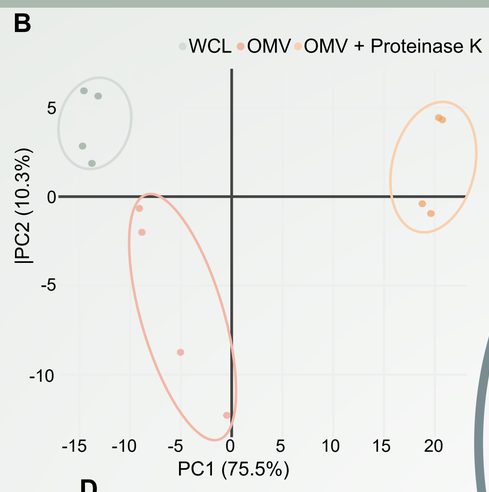


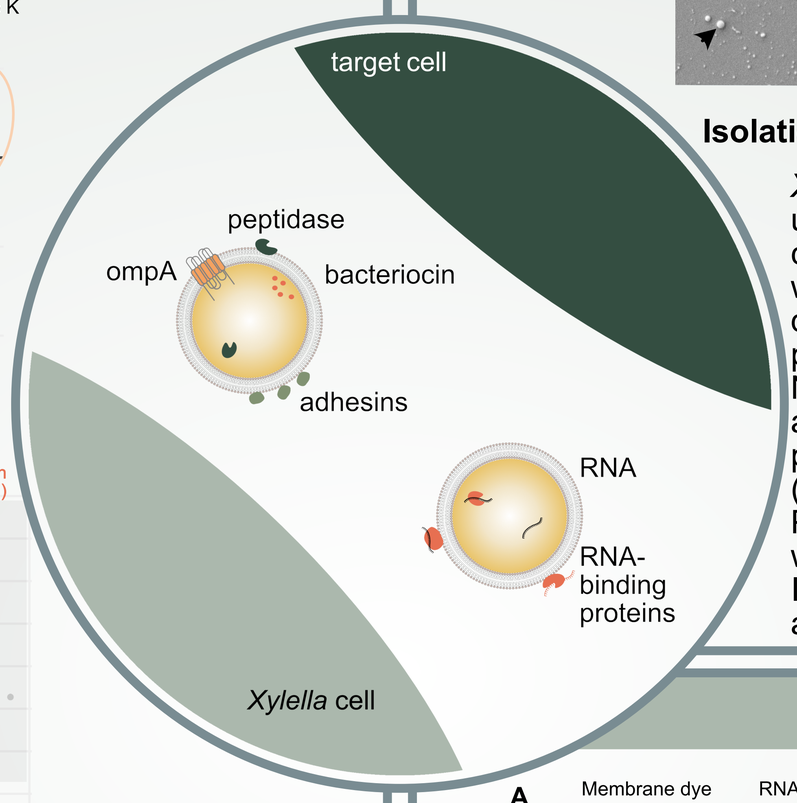
Isolation and Characterization of *Xf*OMVs following MISEV

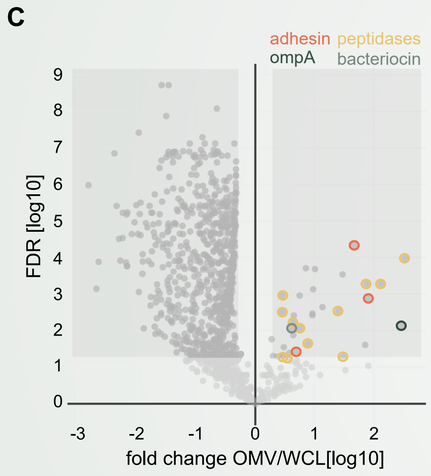
*Xf*OMVs are isolated from axenic cultures via differential ultracentrifugation and further purified via size-exclusion chromatography, as a cellular control we also collected whole cell lysate (WCL) from the same cultures (A). To distinguish between corona of vesicles and internal, protected cargo, OMVs were treated with Proteinase K. Nanoparticle Tracking analysis (NTA) shows consistent size and concentration between treatments with average size profile of 50-300nm of *Xf*OMVs. Surface charge (Zetapotential, ζ) of *Xf*OMVs becomes more negative after Proteinase K - treatment (C). Scanning Electron Microscopy with black arrow heads indicating presence of OMVs (D). Immunoblotting of untreated and treated *Xf*OMVs of OmpA, a homologue of bacterial vesicle marker OprF[2] (E).

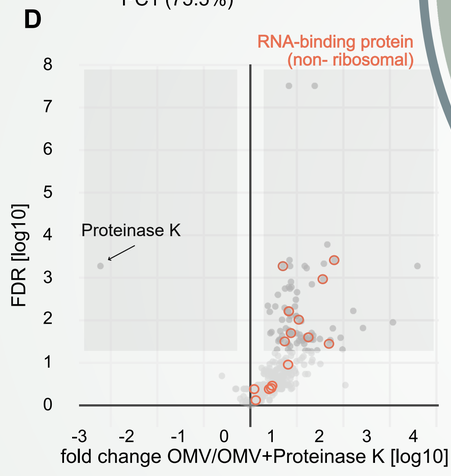
2. PROTEOMICS

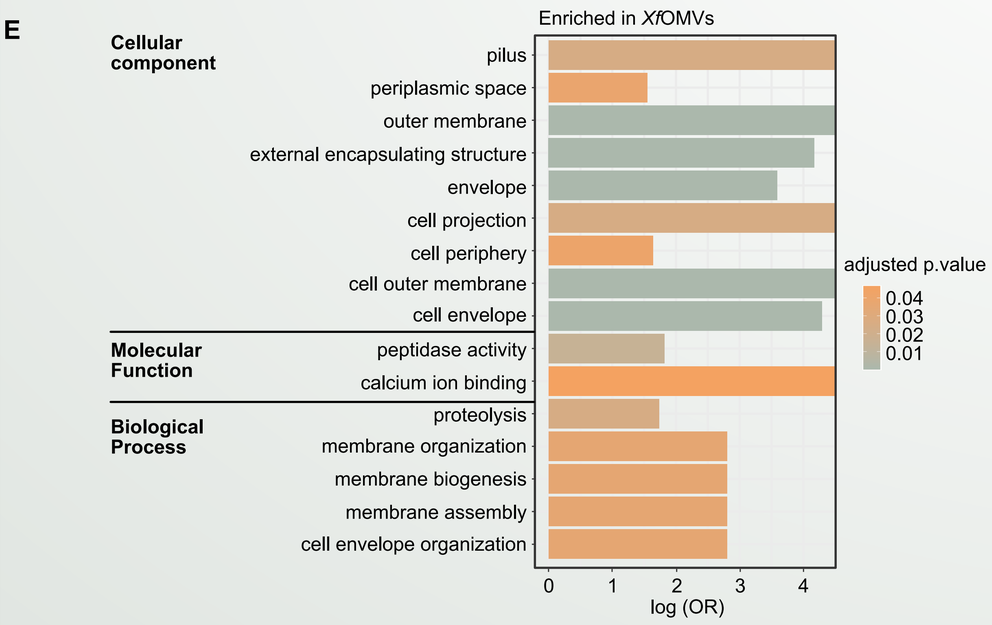








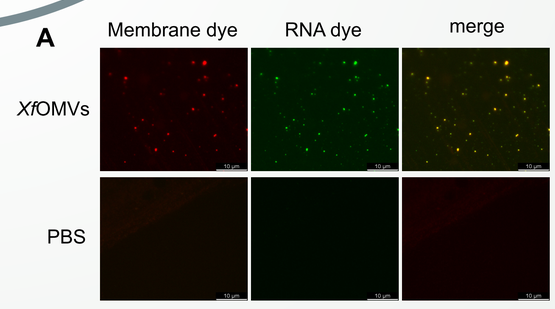


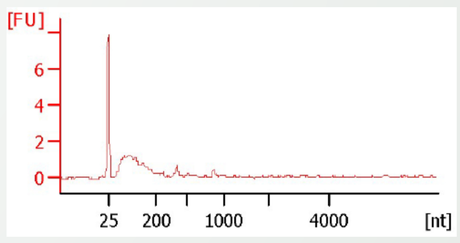


Proteomic analysis identifies bacteriocin, adhesins, peptidases and RNA-binding proteins as cargo of *Xf*OMVs

Proteomic analysis of 3 sample types reveals 301 proteins identified as external OMV cargo (sensitive to Proteinase K) and 77 proteins as internal cargo of OMVs (protected from digestions by Proteinase K). OMV cargo includes 23 proteins which were exclusively detected in OMV-fractions (A). A systematic difference in the proteomes between the 3 sample types can be observed in the principal component analysis (B). Proteins enriched in OMVs compared to WCL include bacterial vesicle marker proteins OmpA (homologue of OprF) and bacteriocin, a potent antimicrobial (C). Enriched proteins in OMVs contain many adhesins, which confirms role of *Xf*OMVs to regulate cell adhesion to surfaces, aiding colonization of plant tissue[1] (C). Additionally, many peptidases are found enriched in OMVs compared to WCL (C). When comparing external and internal cargo of *Xf*OMVs, many (non-ribosomal) RNA-binding proteins can be identified externally attached to OMVs (sensitive to Proteinase K) (D). This includes RNA chaperones and ribonucleases. Gene Set analysis of OMV- enriched proteins indicates an enrichment for outer-membrane structures (CC), an enrichment for proteins involved in proteolysis and membrane- related processes (MF and BP) (E).

3. RNA CONTENT





Small RNA associates with *Xf*OMVs

Staining OMVs with membrane dye FM4-64 and a dye which is only fluorescent when bound to RNA, we can show colocalization of RNA with OMVs (A). RNA can be isolated from OMVs and shows enrichment in small RNAs <200nt (B).

OPEN QUESTIONS

* Do RBPs identified as *Xf*OMV cargo play a role in *Xf* virulence?
* Which RNA sequences do we find in *Xf*OMVs?
* Do bacteria have ckRNAi-mechanisms similar to eukaryotes?
* How does *Xf*OMV cargo change in different growth conditions?

REFERENCES

1 - Ionescu et al., 2014 *PNAS*
2 - Janda et al., 2023 *mBio*

RU5116
DFG Research Unit "exRNA"