Dissecting the (ex)RNA Signature of *Xylella fastidiosa*

Alessa Ruf[1], Patrick Blumenkamp[2], Christina Ludwig[3], Andreas Klingl[1], Andreas Brachmann[1], Alexander Goesmann[2], **Silke Robatzek**[1]

[1]LMU Biozentrum, Munich, Germany, [2]JLU Giessen, Germany, [3]BayBioMS TUM, Munich Germany

Robatzek lab

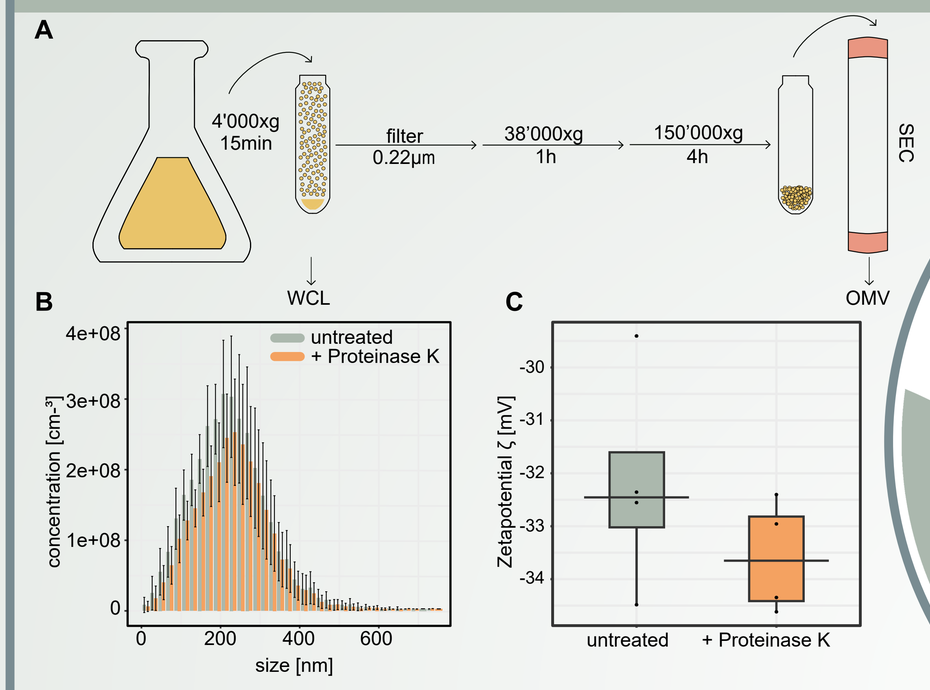
BACKGROUND

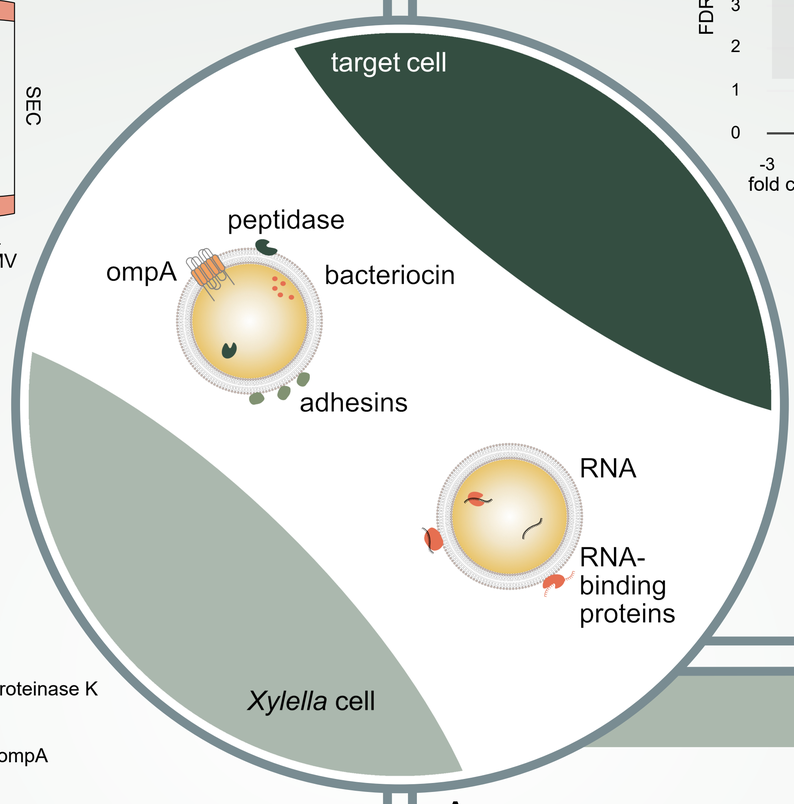
Xylella fastidiosa (*Xf*):

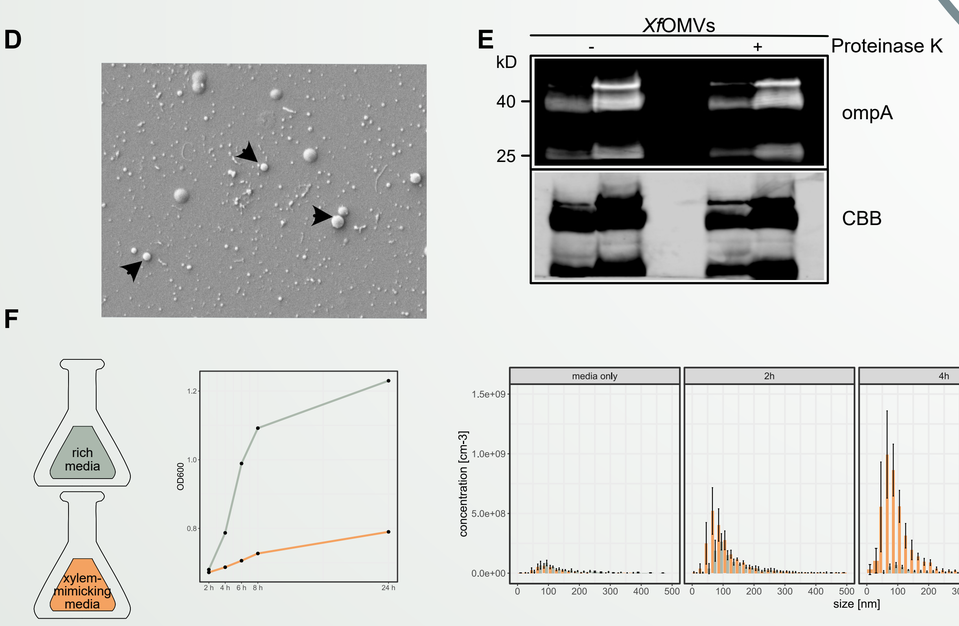
- gram-negative bacterial plant pathogen with many host species
- causes olive quick decline syndrom (OQDS) in Southern Europe
- lacks type-III-secretion system to deliver effectors
- releases ca. 5x more outer-membrane vesicles (OMVs) than closely related species[1]
- little is known about cargo + role 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 virulence of the bacterium 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[2]. 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 (SEC), 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[3] (E). Media shift assays from rich- to xylem-mimicking media shows increased vesiculation in xylem- mimicking media despite reduced growth (F).

OPEN QUESTIONS

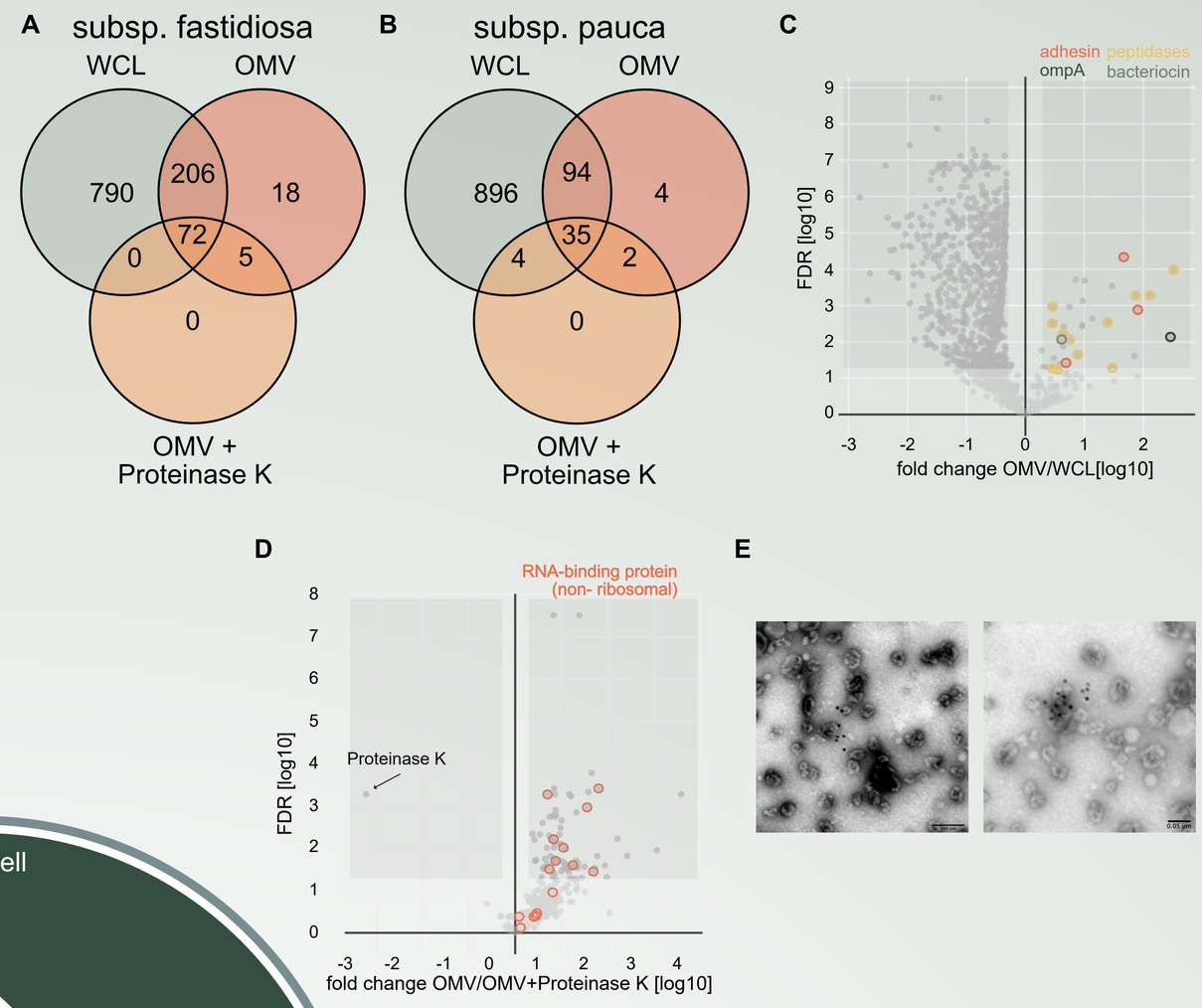
* Do RBPs identified as *Xf*OMV cargo play a role in *Xf* virulence?

* Which RNA sequences do we find in *Xf*OMVs?

* Do we find predicted sRNA in *Xf*OMVs? Do they have targets in host plants?

* How does *Xf*OMV cargo change in different growth conditions?

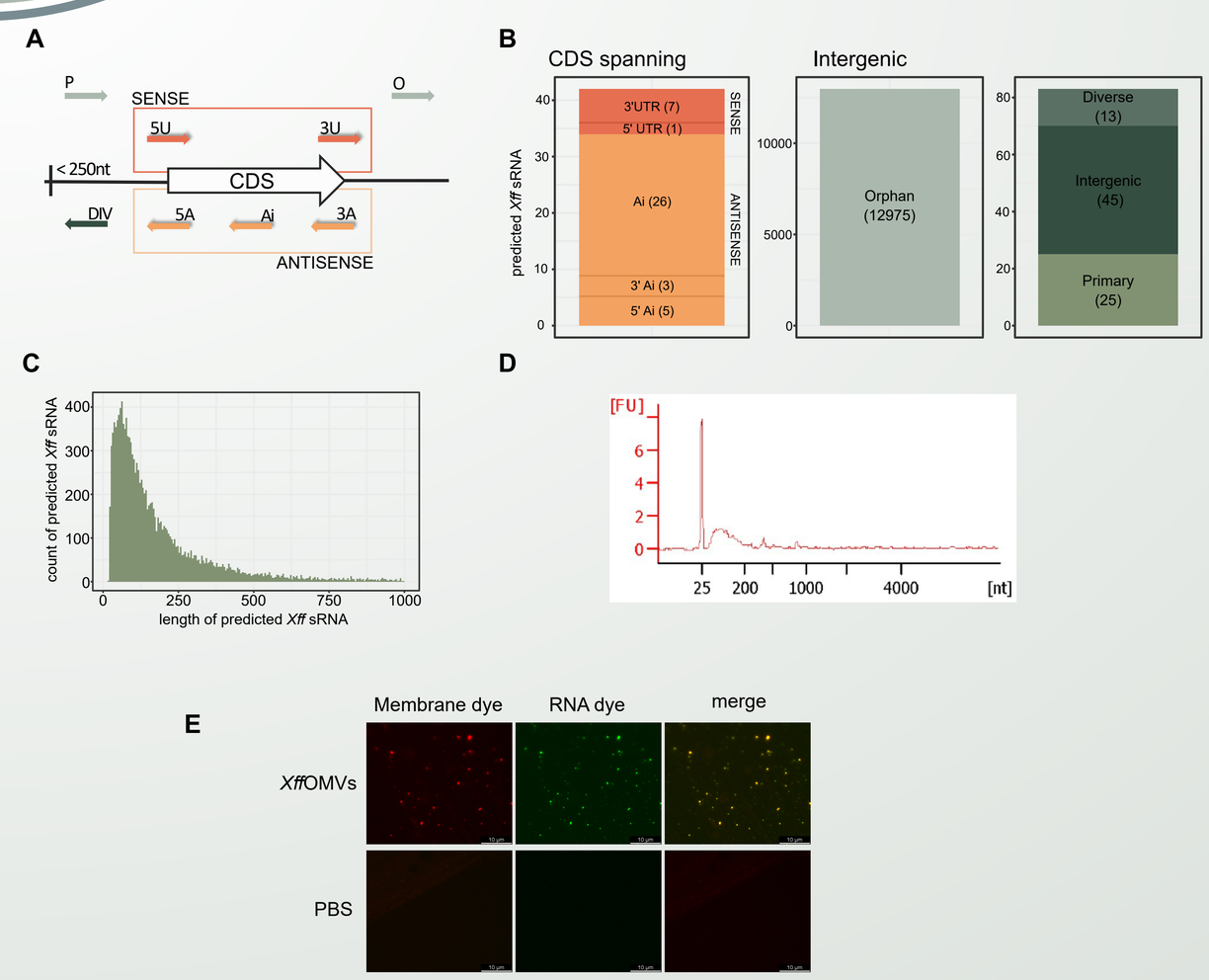
2. PROTEOMICS



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

Proteomic analysis of 3 sample types for two subspecies of *Xf* reveal external protein OMV cargo (sensitive to Proteinase K) and internal protein cargo of OMVs (protected from digestions by Proteinase K). OMV cargo includes proteins which were exclusively detected in OMV-fractions (A). (B). In *Xff*, 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 *Xff*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. Immunogoldstaining using antibodies against one of the identified RBPS, confirms presence of RBPs at *Xff*OMVs (E).

3. RNA CONTENT



Prediction of small RNA repertoire of *Xff* and visualization of small RNA association with *Xff*OMVs

Using the bioinformatical tool APERO[3] paired-end RNAseq data, we could predict sRNAs of *Xff*. Most predicted sRNAs are from intergenic origin but our prediction also contain CDS spanning sense and antisense sRNA with target genes in *Xff* (A and B). Predicted sRNA size ranges mostly from 50- 500nt (C). RNA can be isolated from OMVs and shows enrichment in small RNAs <200nt (B). 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 (E).

REFERENCES

1 - Ionescu et al., 2014 *PNAS*
2 - Ruf et al., 2022 *COPB*
3 - Janda et al., 2023 *mBio*
4 - Leonard et al., 2019 *NucAcRes*

RU5116
DFG Research Unit "exRNA"