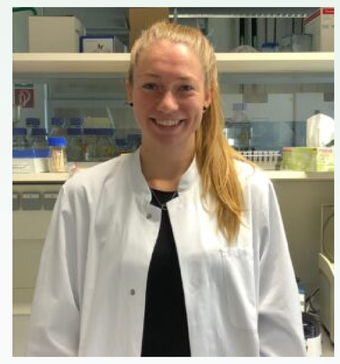
Interactions of bacterial extracellular vesicels with plants

Laura Krassini and Silke Robatzek

Faculty of Biology, Ludwigs-Maximilians-University of Munich, DE

Robatzek lab



BACKGROUND

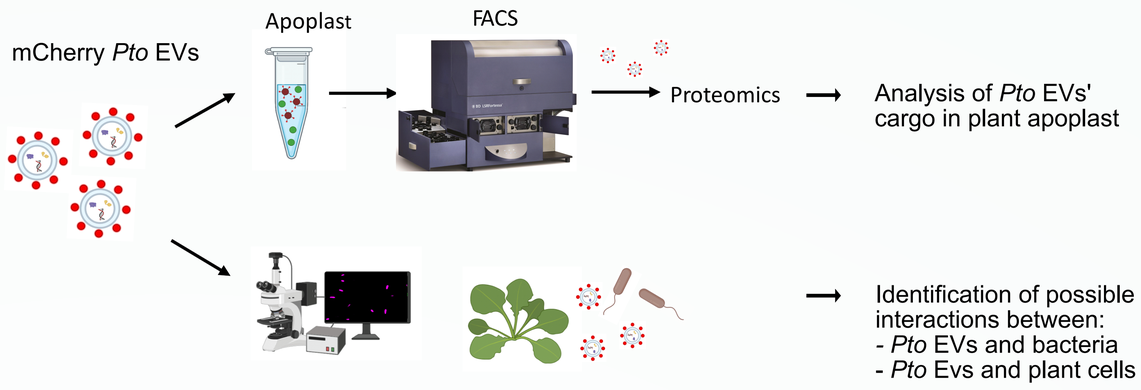
Pseudomonas syringae pv *tomato* (Pto) DC3000:

- A model bacterial plant pathogen colonizing the apoplast and thereby causing disease in tomato and also the genetic model *Arabidopsis thaliana.*
- Vesiculates and releases extracellular vesicles (EVs) in the form of outer membrane vesicles (OMVs) and outer inner membrane vesicles (OIMVs)1.
- The proteins of the corona and cargo of EVs isolated from cultured *Pto* DC3000 suggest roles in nutrition (e.g. iron) and defence against antibiotic molecules1.
- Although the release of EVs was observed *in planta*1, little is known of their role during bacterial infection.

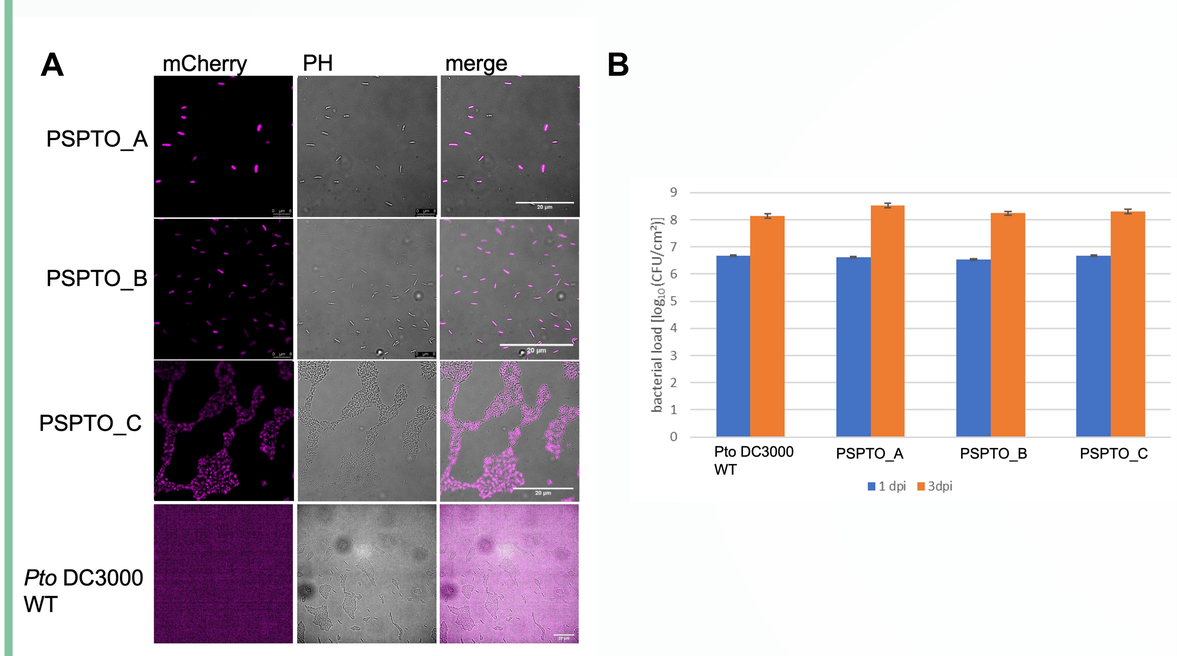
Our research explores proteins of the EV corona and cargo as vesicle biomarkers and to utilize them for studying bacterial EVs *in planta*. We have successfully established fluorescent tagged EV biomarkers and visualized them in apoplastic fluids from infected Arabidopsis. One aim is to describe the *Pto* DC3000 EV protein composition *in planta* and to identify potentially plant-interacting proteins.

METHODOLOGY

Fluorescent EV marker *Pto* marker line: mCherry fusion with outer membrane protein

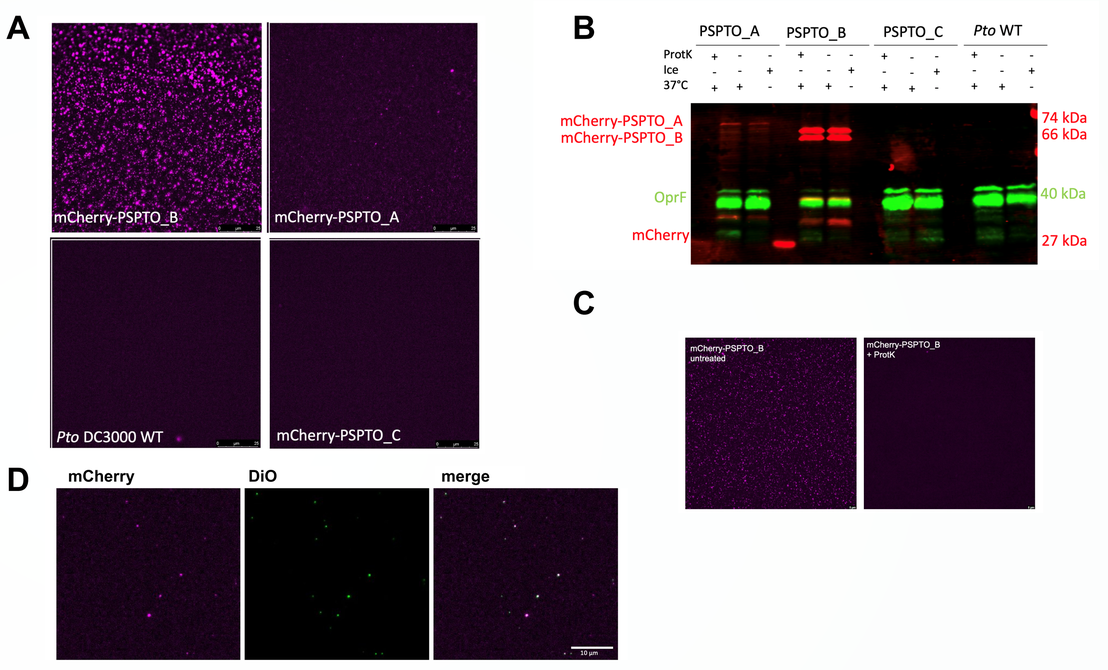


1. mCherry tagging of EV proteins in *Pto* DC3000



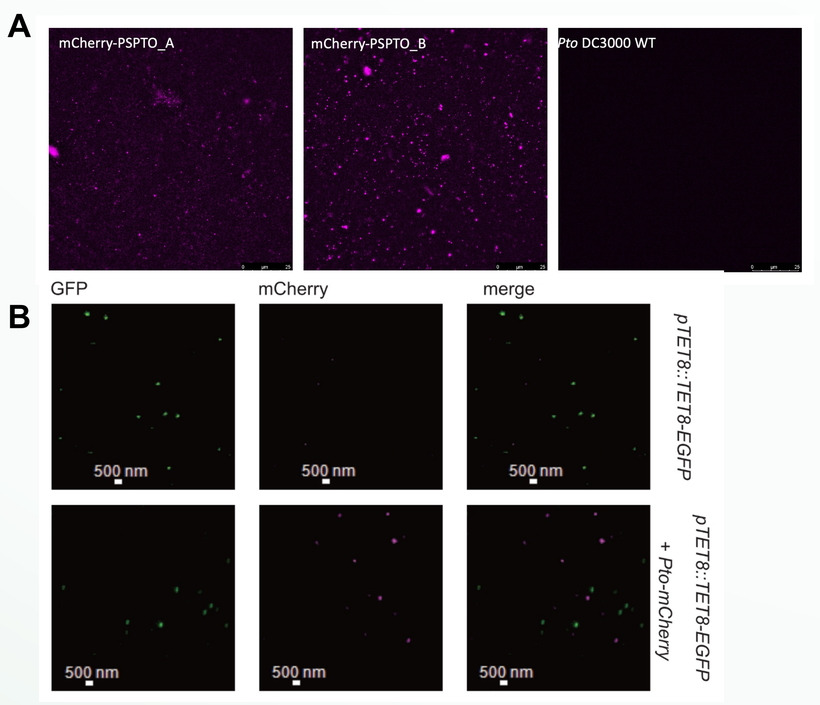
Three candidate EV biomarker proteins (¹) were selected for mCherry tagging and expressed in *Pto* DC³⁰⁰⁰. (A) Fluorescence microscopy imagevs of transformed bacteria. (B) Transformed bacteria do not differ in their virulence when infected in Arabidopsis.

2. Detection of mCherry- EV biomarker *in-vitro*



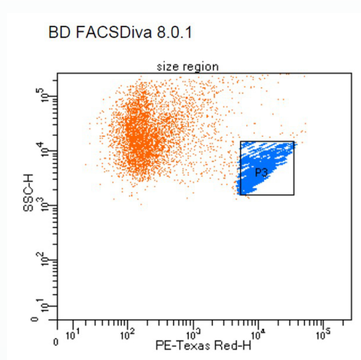
EVs collected from the three candidate EV biomarker transformed *Pto* DC³⁰⁰⁰ strains can be detected by fluorescence microscopy (A) and immunoblot analysis (B). PSPTO_B shows the strongest signal, sensitive to Proteinase K (ProtK) treatment (C) co-labelling with the membrane dye DiO (D).

3. Detection of mCherry- EV biomarker *in-planta*



mCherry-tagged *Pto* DC³⁰⁰⁰ EV biomarkers can be detected in apoplastic wash fluids (AWF) from infected Arabidopsis. Fluorescence microscopy images (A, C) and immunoblot analysis (B, D) of filtered AWF from infected WT Arabidopsis (A, C) or the plant TET⁸-GFP EV marker line (B) or in comparison with *bak¹-⁵* mutants (D). Bacterial PSPTO_B-positive EVs are distinct from plant TET⁸-positive EVs.

4. Sorting of mCherry- EVs



Tube: HP

Population	#Events	%Parent	%Total
All Events	10,173	####	100.0
size region	5,400	53.1	53.1
P3	428	7.9	4.2

FACS sorting of mCherry-PSPTO_B reveals a distinct population of EVs (blue square).

OUTLOOK

- To create deletion mutants of the selected EV biomarker in *Pto* DC3000 and study its phenotype
- To isolate mCherry-positive *Pto* Dc3000 EVs from apoplastic fluids of infected Arabidopsis
- To determine the EV corona and cargo proteome as well as identify plant-interacting proteins
- To measure *Pto* DC3000 EVs for metal ions

REFERENCES & ACKNOWLEDGEMENT

References:
1 Janda et al., Biophysical and proteomic analyses of Pseudomonas syringae pv. tomato DC3000 extracellular vesicles suggest adaptive functions during plant infection, (2023), mBio

Acknowledgments:
We thank the SFB924 for funding and the FACS facilty at the LMU Munich BMC.
Experiments with Tet8 were performed together with Eliana Mor.
Part of Figures were createdv with BioRender.com.