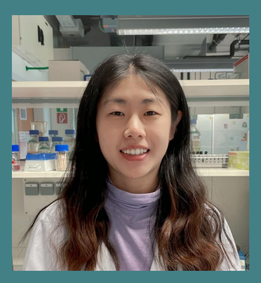
The relationship between *Xylella fastidiosa* and microbiome



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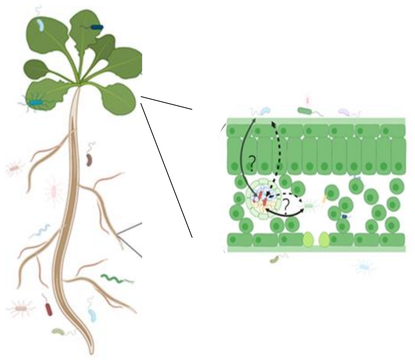
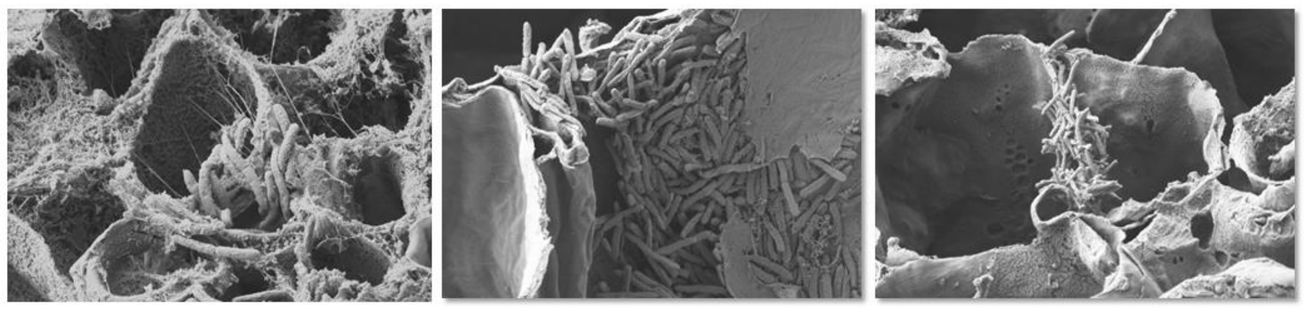
Background

Xylella fastidiosa is the causal agent of important crop diseases and is transmitted by xylem sap-feeding insects. The bacterium colonizes xylem vessels and can persist with a commensal or pathogen lifestyle in more than 500 plant species. Several studies have begun to characterize microbiome diversity in crops infected by *X. fastidiosa*, describing changes in the xylem microbiome composition during disease. Differences were detected in both the richness and diversity of the microbial communities associated with resistant and susceptible host plants[1]. *Arabidopsis thaliana* (*At*) as a genetic model plant could be successfully colonized by *X. fastidiosa* (Fig 1). We use *At* as a host to address the questions how *X. fastidiosa* influence microbiome assemblages and *vice versa* how the microbiome influences the outcome of pathogen infection.

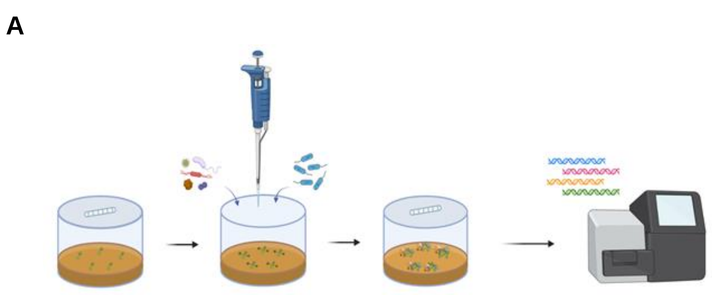
Figure 1: Colonization of *Xylella fastidiosa* subsp. *fastidiosa* (*Xff*) in *Arabidopsis*





Scanning electron micrographs showed the presence of *Xff* colonizing in xylem vessels of *Arabidopsis* Col-0 seedlings at 3 dpi.

Figure 2: *Xff* modulates microbiome composition

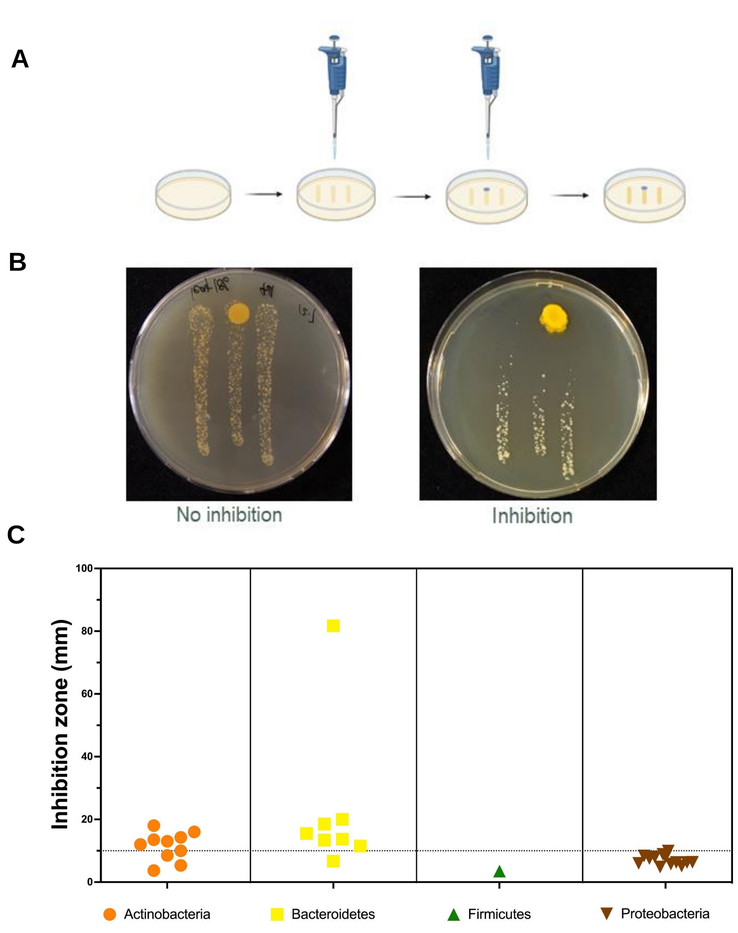


(A) A gnotobiotic system was used to co-inoculate *Xff* and the synthetic community (SynCom) consisting of 62 *At*-LSPHERE strains in germ-free *At* seedlings. The above ground parts of the plants were harvested to profile the bacterial communities.

(B) Microbiome richness was slightly reduced after *Xff* infection.

(C) The relative abundance of microbiome was altered by *Xff*, which Bacteroidetes were enriched.

Figure 3: *Xff* growth is inhibited by 32 *At*-LSPHERE isolates

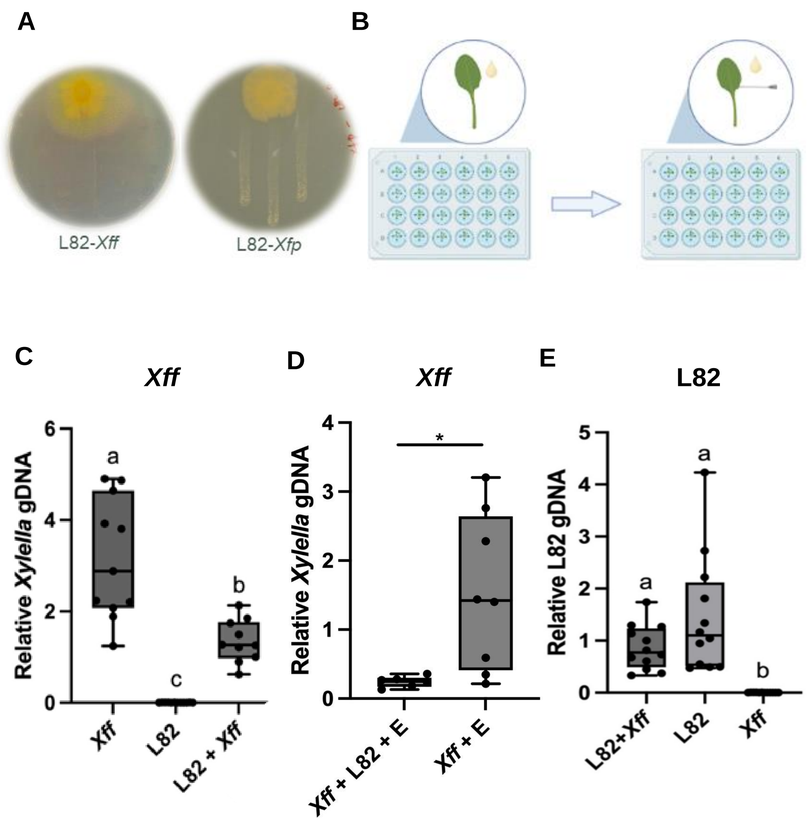


(A) 224 *At*-LSPHERE isolates were tested to identify antagonistic bacteria that are potentially deployable as biocontrol agents against *Xff*. *Xff* was streaked on plates for 4 days then dropped the microbiome isolate. The inhibition zone was measured to quantify *Xylella* growth.

(B) Pictures shows the phenotype of inhibition and no inhibition on plates under lab condition.

(C) 32 isolates have different degrees of antagonistic activities against *Xff*, comprising 10 from Actinobacteria, 8 from Bacteroidetes, 1 from Firmicutes and 13 from Proteobacteria.

Fig. 4: Flavobacterium sp. Leaf82 (L82) inhibits *X. fastidiosa in vitro* and *in vivo*



(A) A strain from Bacteroidetes phylum, L82, showed completely inhibitory effect against *Xff* growth on the plate, which is the strongest among all the *At*-LSPHERE isolates. It also performed inhibition effect against *Xylella fastidiosa subsp. pauca* (*Xfp*).

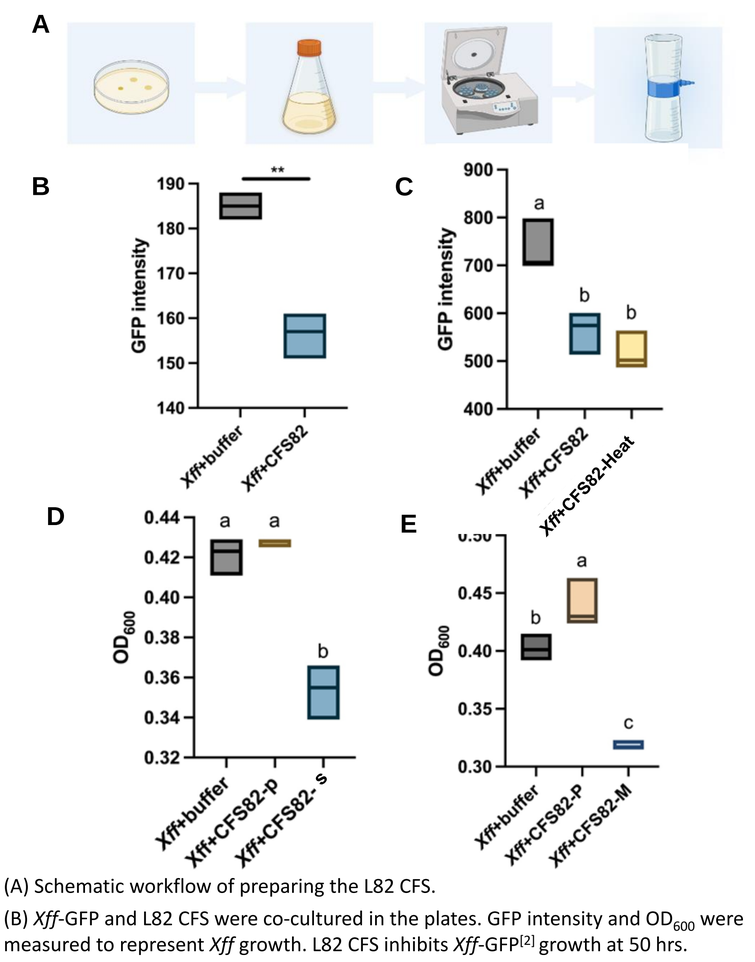
(B) Scheme of plate-based seedling system. Plants at 10-11 days old were inoculated with L82 by dropping bacteria suspension on the leaves. 20 days old plants were infected with *Xff* by pricking the junction of leaf and petioles.

(C) Quantitative real-time PCR (qPCR) was used to quantify bacterial abundance in *At* seedlings. The results showed that *Xylella* was less abundant in the plants inoculated with L82 (L82+*Xff*).

(D) To quantify endosphere *Xff*, plants were washed with ethanol. The results showed lower *Xff* abundance in L82 treated samples (L82+*Xff*+E).

(E) The abundance of L82 is not influenced by *Xff*.

Figure 5: Cell free supernatants (CFS) of L82 inhibits *Xff* growth



(A) Schematic workflow of preparing the L82 CFS.

(B) *Xff*-GFP and L82 CFS were co-cultured in the plates. GFP intensity and OD_{600} were measured to represent *Xff* growth. L82 CFS inhibits *Xff*-GFP[2] growth at 50 hrs.

(C) We further tested the properties of the antibacterial active substances in L82 CFS. L82 CFS were heated at 100 ℃ for before treatment. The data revealed heated CFS still had inhibition effect, which indicates that it has thermal stability.

(D) Fractions of the L82 CFS were prepared by Ultracentrifugation. Two fractions were tested for the inhibition. Fraction of pellet part (CFS82-p) lost the inhibition activity, indicating that the molecules is not vesicles.

(E) We further prepared the L82 CFS by filtering supernatant part (CFS82-s) through a 3 kDA filtration falcon tube. The inhibition effect was remained in the <3kDA fraction (CFS82-M), which demonstrated that the antibacterial active substances might be a metabolite.

Outlook

Conclusion:

- *Xff* alters microbiome composition.
- Inter-bacterial interactions determine the ability of *Xff* growth *in vitro* and *in planta*

Open questions:

- How does *Xff* shape the microbiome composition?
- What is the molecular mechanism of the inhibition by different microbiome isolates?
- Is plant immune system involve in the microbiome-*Xylella* interaction?

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- Method scheme were created with BioRender.com

References

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[2] Newman Karyn L., et al. "Use of a Green Fluorescent Strain for Analysis of *Xylella fastidiosa* Colonization of *Vitis vinifera*." Appl Environ Microbiol (2003).