XYLENCER - Enhanced bacteriophage therapy against *Xylella fastidiosa*

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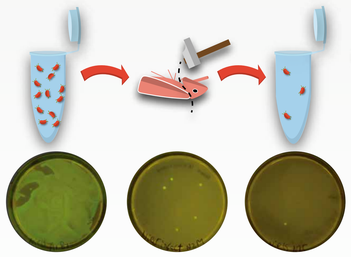
Throughout the Mediteranean, olive trees are drying out, but not for lack of water[1,2]. ***The cause?*** *Xylella fastidiosa*, a plant pathogen bacterium that is spread by insects, killing plants where it goes[3]. A cure to *X. fastidiosa* is essential.
Xylencer uses phage therapy to treat *X. fastidiosa*, bringing us closer to a cure for it.

DETECTION

To automate detection of *X. fastidiosa*, we designed a plant mimic. Insects carrying *X. fastidiosa* that feed on the plant can thereby transfer the bacterium. This can be detected using Loop-mediated isothermal AMPlification (LAMP).

LAMP amplifies DNA efficiently at a constant temperature. The LAMP reaction lowers pH, which is used as an indicator.

Positive (left) and negative (right) result LAMP test.



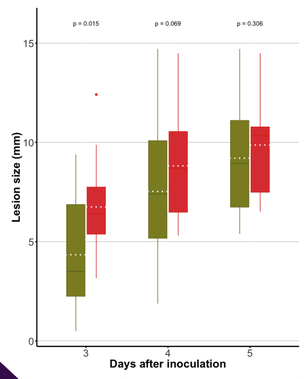
Bacteria can be transmitted by insects when feeding on the plant mimic.

> Detection of *X. fastidiosa* is a major problem
> *-Dr.ir. Martijn Schenk NVWA*

REMEDIATION

The Xylencer phage genome codes for Pathogen Associated Molecular Patterns (PAMPs), which are released upon lysis, triggering plant immunity.

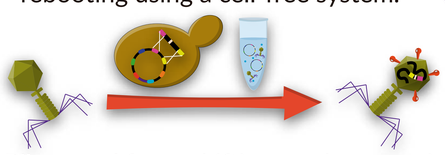
We injected *Xanthomonas*-infected plants with flg22. Disease symptoms were measured in the days after (days 3, 4 and 5).



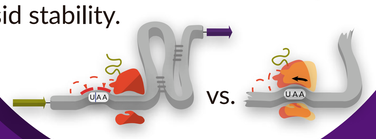
***Lesion size in* Brassica oleracea.** *Lesion size after inoculation with flg22 (green) or control (H_2O) (red).*

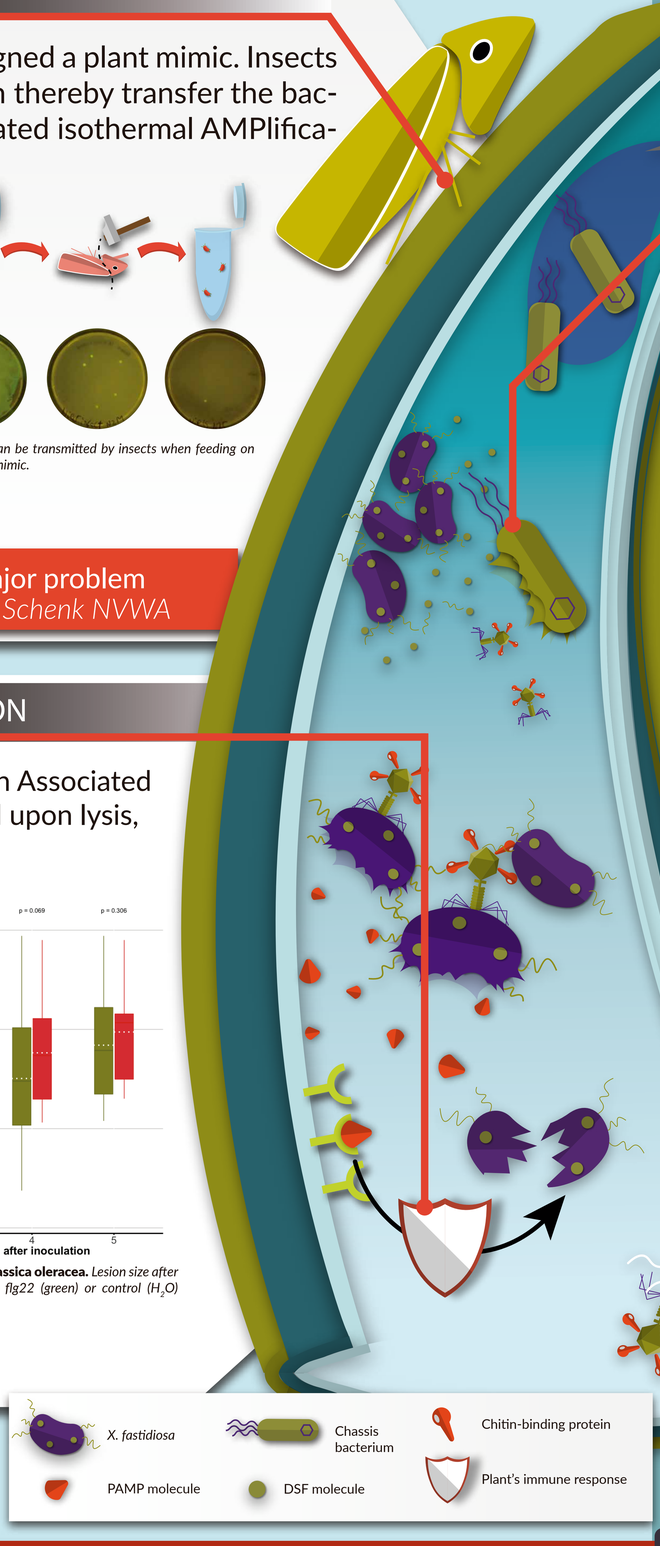
PHAGE ENGINEERING

Yeast-based genome assembly and rebooting using a cell-free system.



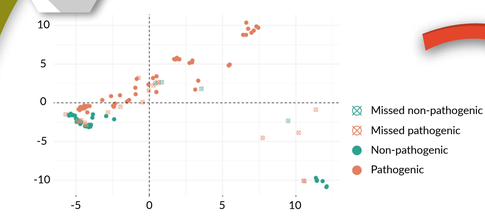
Ribosomal frameshifting produces a mix of WT and fusion protein, increasing phage capsid stability.



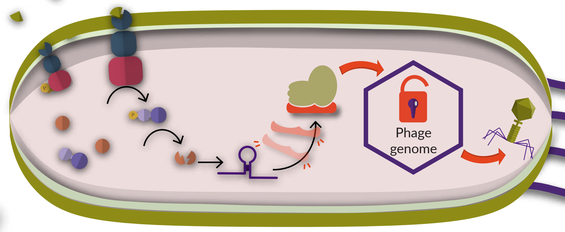


DELIVERY

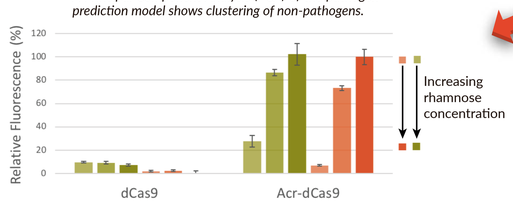
A chassis bacterium carries the phage on a plasmid, repressed by dCas9. When *X. fastidiosa*'s quorum sensing molecules are detected, the repression is lifted by anti-CRISPRs (Acrs), allowing the phage to be produced.



A Principal Component Analysis (PCA) of the pathogen prediction model shows clustering of non-pathogens.

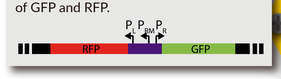


The chassis bacterium includes a DSF sensing circuit, which controls Acr expression through a riboswitch. Acrs then lift dCas9 repression of the phage, leading to production of phages.



(Acr-)dCas9 targeting gfp and mrfp. *Fluorescence of dCas9 repressed RFP (red) and GFP (green) at different levels of Acr induction.*

Lambda phage promoters PL, PR and PRM were targeted using dCas9 (repressing GFP and RFP). Acrs were then induced at different levels, leading to the expression of GFP and RFP.

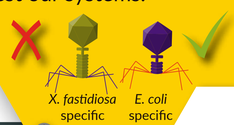


> Temperature and UV-light degrade phages
> *-Prof. dr. Britt Koskella UC Berkeley*

BIOSAFETY

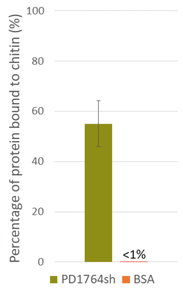
Biocontainment strategy: timed kill switch incorporated in the chassis bacterium.

Biosecurity: we used model organisms to test our systems.



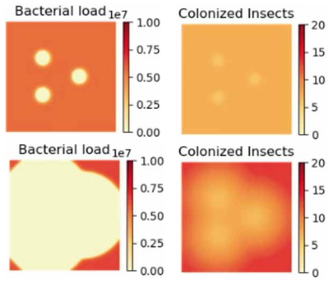
SPREAD

To be able to reach a large number of plants with the cure, chitin-binding (CB) proteins are fused to the Xylencer phage capsid. This will allow the phage to spread from plant to plant *via* insects.



Percentage of CB protein PD1764sh and control BSA bound to chitin.

The chitin-binding ability of the proteins was tested by pelleting chitin with isolated CB protein. Unbound protein concentrations were measured using a Bradford assay.



> Plant viruses are spread by insects
> *-Dr. Ir. René van der Vlugt, Plant Virology WUR*

CONCLUSIONS

- Successful *X. fastidiosa* detection.
- Machine learning identification of a chassis bacterium.
- Regulation of a synthetic Lambda circuit by dCas9 and Acrs.
- Treatment of *Xanthomonas* infection using PAMPs.
- Effective chitin-binding proteins and verified capsid protein fusions.

ATTRIBUTIONS

We want to thank our team of iGEM supervisors, the departments of Microbiology & Systems and Synthetic Biology and all stakeholders that have helped us with our project!

1. Strona, G., Carstens, C. J., & Beck, P. S. A. (2017). Network analysis reveals why Xylella fastidiosa will persist in Europe. Scientific Reports, 7(1), 71.
2. EFSA. (2019). Xylella fastidiosa - EU legislation.
3. CABI. (2019). Xylella fastidiosa (Pierce's disease of grapevines) (Datasheet).