Transient gene silencing - a step forward in identifying novel pathogenicity factors in the late blight pathogen, *Phytophthora infestans*

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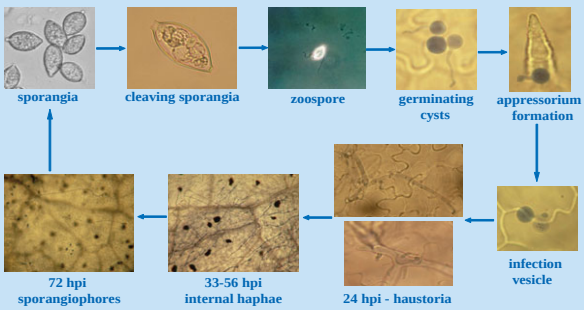
Background

Phytophthora infestans causes late blight of potato and tomato. It produces several different cell types prior to host penetration and during the early stages of infection.

Phytophthora infestans infection cycle



Appressoria, structures formed just prior to infection, are likely to contain many transcripts required for successful penetration of the host and establishment of a compatible interaction.

Strategy

High-throughput screen for gene function in *P. infestans*

50 genes up-regulated in germinated cysts, appressoria and *in planta* secreted proteins:

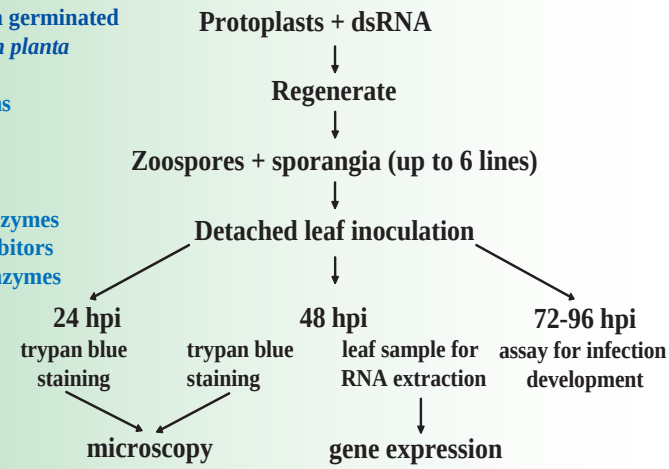
- 9 novel secreted proteins
- 21 RxLR class effectors
- 1 adhesion
- 1 PAMP
- 2 cell wall degrading enzymes
- 3 cysteine protease inhibitors
- 2 ROS detoxification enzymes

structural proteins:

- 2 membrane proteins
- 3 transporters

2 enzymes affecting development

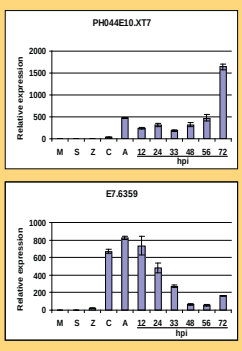
4 other



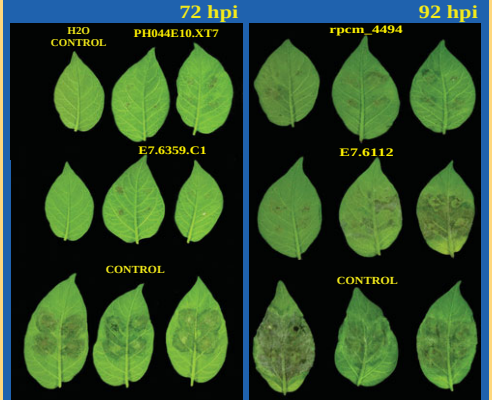
Results

Novel secreted proteins and RxLR effector proteins

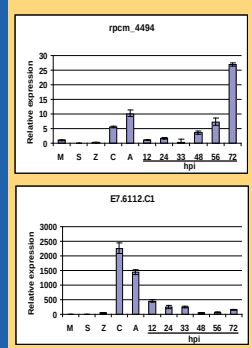
Secreted protein gene expression profiles in pre-infection stages and *in planta*



Silencing these genes affects pathogenicity

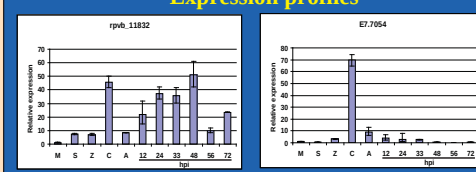


RxLR effector gene expression profiles in pre-infection stages and *in planta*

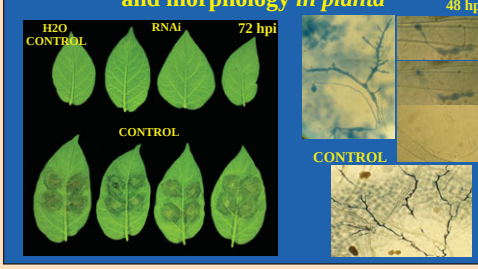


Palmitoyl-protein thioesterase-2 like

Expression profiles

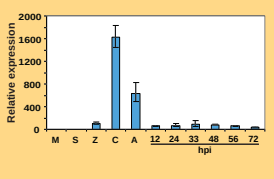


Silencing rpvb_11832 affects pathogenicity and morphology *in planta*



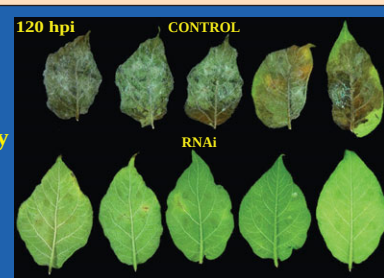
Oomycete-specific haustorial membrane protein, PiHMP1

Expression profile in pre-infection stages and *in planta*

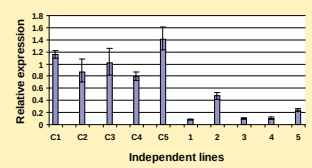


***PiHMP1*-*mRFP* fusion**

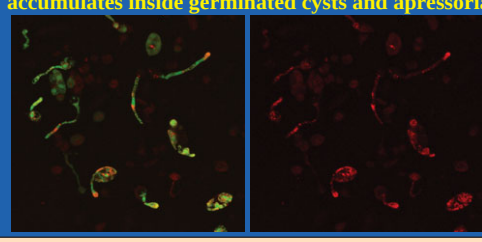
Silencing *PiHMP1* affects pathogenicity



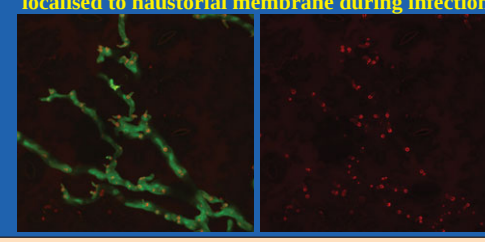
Relative expression levels of *PiHMP1* in control and silenced lines



accumulates inside germinated cysts and apressoria



localised to haustorial membrane during infection



Conclusions

Transient RNAi is effective for identifying genes with a major role in pathogenicity. RNAi can inform hypotheses for downstream analysis of pathogenicity in *P. infestans*.

Both formation of functional pre-infection structures and pathogenicity require the action of many genes.

Future work

Localisation of structural proteins by translational fusion to fluorescent proteins, and subsequent observation by confocal microscopy.

Acknowledgements

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