



Conserved putative effectors across four *Verticillium dahliae* strains isolated from strawberry (*Fragaria x ananassa*).

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Fig.1: Strawberry genotypes with susceptibility (left) and resistance (right) to *Verticillium dahliae*.

Sequencing & Bioinformatics

Next generation sequencing (*MiSeq*)

- Quality trimming of reads (*fastq-mcf*).
- Assembly of genomes (*Velvet*) using different hash lengths.
- Optimum assembly selected

Blast known *V. dahliae* pathogenicity genes listed in PHI-base to the assembled contigs (*tBLASTn*).

Identification of gene models (*Augustus*)

- Discovery of proteins containing signal peptides (*SignalP*), small cysteine rich sequences (*SSCP*) and host uptake motifs (*RxLR*).
- Blast candidate gene models to determine homology of candidate effectors to known pathogenicity genes in PHI-base (*BLASTp*).

Figure 2: NGS pipeline for identification of pathogenicity genes & effectors in *V. dahliae*

<i>Verticillium dahliae</i> isolate	VdLs.17	12251	12253	12158	12161
Genome size (Mb)	32.9	32.3	31.9	31.7	32.0
Number contigs >1kb	1525	1214	1842	1116	1082
Number in N50	217	193	295	176	159
N50 kb	43.31	52.42	32.89	56.26	62.34

Table 1: Genome assembly statics for VdLs.17 (isolated from lettuce) and four *Verticillium dahliae* isolated from Strawberry.

<i>Verticillium dahliae</i> isolate	12251	12253	12158	12161
Gene models	10731	10822	10478	10460
Secreted proteins	1269	1263	1240	1231
Small secreted cysteine rich	41	43	38	40
Secreted RxLR's	26	26	21	21
Small secreted cysteine rich RxLR's	2	2	2	2

Table 3: The number of gene models, gene models that contain secreted proteins, small secreted cysteine rich proteins (SSCP), secreted RxLR's and SSCPs containing RxLRs in four *Verticillium dahliae* isolates from strawberry.

Introduction

Verticillium dahliae is a global and major soil borne disease affecting approximately 300 different plant species. There are few remaining options for effective control of *V. dahliae*, due to the phasing out of chemical fumigants under EU legislation. In the UK over 40% of strawberries are now grown in substrate such as coir and peat to avoid soil borne plant pathogens, however the development of commercially viable, disease resistant cultivars will allow the re-introduction of strawberry production into soil. The identification of *V. dahliae* effectors will allow the characterisation of plant-pathogen interactions and assist effector informed breeding to ensure the production of robust disease resistant cultivars.

Methods

- Paired end 250bp sequence data was generated for four *V. dahliae* isolates (12251, 12153, 12158 & 12161) which exhibit variation in virulence on strawberry. Data was assembled and BLASTed to PHI-base as outlined in Figure 2.

Results

- **Assembly:** The four *V. dahliae* strains isolated from strawberry show a similar genome size to isolate VdLs.17 (Table 1). Three out of four assemblies show a higher N50 value than the VdLs.17 assembly indicating high quality draft assemblies.
- **Presence of known pathogenicity genes:** *VMK1*, *VdSge1* and *VGB* pathogenicity genes are present in all four strawberry isolates^{1,2,3}. Isolates 12251 and 12253 contain unaltered NLP1 and NLP2 pathogenicity genes, however, Isolates 12158 and 12161 contain NLP1 and two with amino acid sequence alterations⁴. Ave1 is a truncated protein in all isolates therefore none of the isolates have a functional Ave1 amino acid sequence (Table 2)⁵.

- **Identification of candidate effectors:** The gene models (Table 2) for each isolate were assessed for candidate effector sequences. Across isolates, 11.8(±0.03) % of gene models contained secretion signals. Further analysis determined that of the small secreted cysteine rich proteins (SSCP) identified (Table 2), five unique SSCP are found in isolate 12253, two in isolate 12161 and one in each of the remaining isolates. All isolates contained a SSCP showing partial homology to MHP1 (E = 3X 10⁻³⁸, 102 % length of sequence, 66% identical) a Class II hydrophobin. MHP1 was expressed in *Magnaporthe grisea* during infection of rice and absence of MHP1 resulted in reduced virulence⁶. The remaining identified SSCP's did not show sequence homology to existing pathogenicity genes in PHI-base. Two of the SSCP's contained RxLR motifs and were found in all four isolates. One of these SSCP's is homologous to the *Verticillium alfalfae* *VaMs.102* which reported as a Class II Hydrophobin. In *Cryphonectria parasitica*, a class II hydrophobin was required for stromal pustule eruption from the bark⁷.

- The secreted proteins containing RxLR motifs in the first 110 bases of the start codon (Table 1) were BLASTed against PHI-base. The FGSG_13509 gene involved in maintaining virulence in *Fusarium graminearum* was present in all isolates apart from 12253⁸. A Gin4-like (CDC) homolog involved in maintaining virulence in *Fusarium graminearum* was present exclusively in isolate 12261⁸. The gene *PTH11* involved in aspersorium formation in *Magnaporthe grisea* was identified in isolates 12253 and 12251⁹.

Conclusions & Future work

- The lack of Ave1 in all isolates of *V. dahliae* suggests that there may be a functional *Ve1* ortholog in *F. x ananassa*
- Variation between putative effectors and identified pathogenicity genes exists between four strains of *V. dahliae* isolated from strawberry.
- Effector cloning and transient expression on differentially susceptible strawberry lines will augment the comparative genomics of *V. dahliae* isolates and assist the determination of the relationship between isolate effector complement, relative pathogenicity and host resistance.

Pathogenicity gene	<i>Verticillium dahliae</i> isolate			
	12251	12253	12158	12161
Ave1	-	-	-	-
NLP1	✓	✓	(A-5-T) (I-15-S) (T-79-P) (R-80-Q)	(A-5-T) (I-15-S) (T-79-P) (R-80-Q)
NLP2	✓	✓	(R-67-P)	(R-67-P)
VGB	✓	✓	✓	✓
VMK1	✓	✓	✓	✓
VdSge1	✓	✓	✓	✓

Table 2: Presence of known *V. dahliae* pathogenicity genes in *V. dahliae* strains isolated from strawberry. ✓ Present: sequence identical X Not present, - Truncated protein, () change in amino acid sequence at denoted positions.

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