An Ancestral Oomycete Locus Contains Late Blight Avirulence Gene *Avr3a*, Encoding a Protein that is Recognised in the Host Cytoplasm

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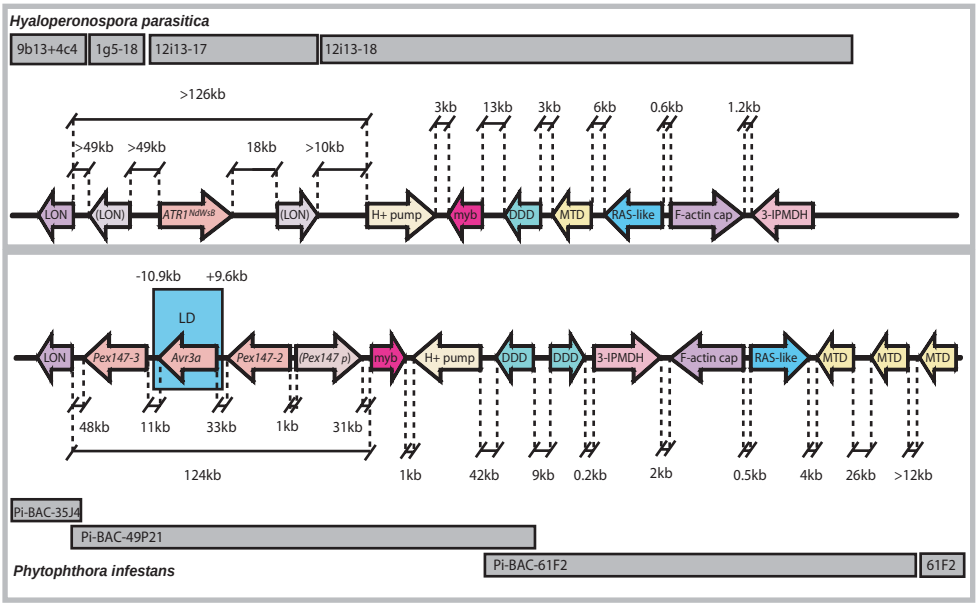
INTRODUCTION:

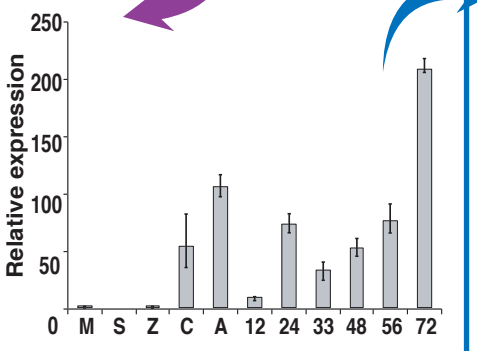
The oomycete *Phytophthora infestans* causes late blight,the potato disease that precipitated the Irish famines in 1846 and 1847. It represents a re-emerging threat to potato production and is one of over 70 species which are arguably the most devastating pathogens of dicotyledonous plants. Nevertheless, little is known about the molecular bases of pathogenicity in these algae-like organisms, or of avirulence molecules that are perceived by host defences. Disease resistance alleles, products of which recognise corresponding avirulence molecules in the pathogen, have been introgressed into the cultivated potato from a wild species, *Solanum demissum*, and *R1* and *R3a* have been identified. We used association genetics to identify *Avr3a*, and show that it encodes a protein that is recognised in the host cytoplasm, where it triggers *R3a*-dependent cell death (Armstrong *et al* (2005) *Proc. Natl. Acad. Sci (USA)* 102:7766-7771.

RESULTS:

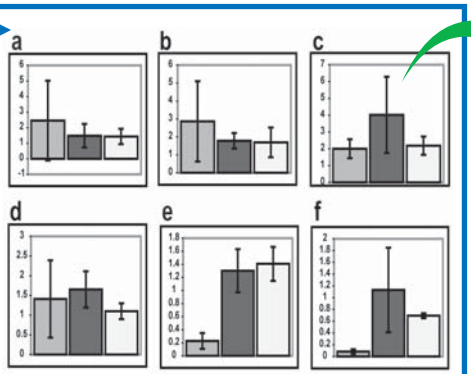
We used association genetics to show that *Pex147* (encoding a 147 amino acid secreted protein) was likely to be *Avr3a. Pex147* was PCR amplified from 55 virulence-tested *P. infestans*. Three SNPs were found, changing amino acids S19C, E80K and M103I. The SNPs revealed two alleles correlated 100 % with virulence phenotype on cv. Pentland Ace, containing R3a. The C19 K80 I103 (C-K-I) allele was associated with avirulence, while virulent isolates were homozygous for the S19 E80 M103 (S-E-M) allele. BACs containing *Pex147* were sequenced to reveal two paralogues of *Pex147* (*Pex147-2* and *Pex147-3*).



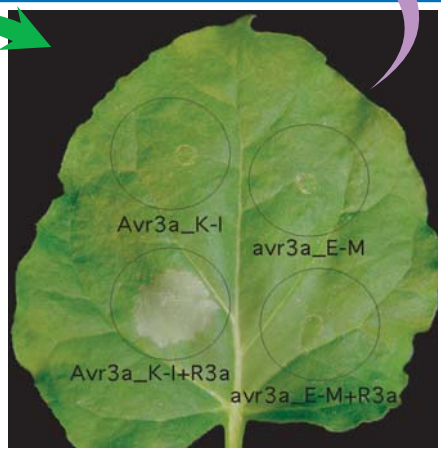
The *Avr3a locus* revealed coding sequences (CDSs) similar to CDSs flanking *ATR1NdWsB* in *Hyaloperonospora parasitica*. MTD, a RAS-like protein, F-actin capping protein and 3-IPMDH were co-linear in the loci. *ATR1NdWsB* and *Avr3a* share little sequence similarity, but are in similar relative locations. Within these co-linear loci, the avirulence genes have evolved differently. In *H. parasitica*, considerable allelic variation was observed in *ATR1NdWsB.* In *P. infestans*, gene duplication and divergence has generated variation in *Avr3a*-like sequences and numerous synonymous and non-synonymous SNPs distinguish these genes.



Expression of *Pex147* was readily detectable in pre-infection (sporangia, zoospores, germinating cysts, and appressoria) and infection (susceptible potato cv. Bintje, 12, 24, 33, 48, 56 and 72 hpi) stages. *Pex147* was up-regulated >100-fold in appressoria and showed elevated levels of expression throughout infection, with an early peak of expression at 24 hpi, in the biotrophic phase of infection, and >200-fold elevation of expression at 72 hpi, in the necrotrophic phase of the interaction.



Truncated E-M (virulent) and K-I (avirulent) alleles of *Pex147* were co-expressed with a vector expressing gfp, as a marker of cell vitality, in potato genotypes lacking an *R* gene (cv. Bintje), or containing *R3a, R3b, R1, R2 and R10*. There was a 5-fold reduction in GFP fluorescence in Pentland Ace (R3a) co-bombarded with the truncated K-I sequence, indicating a reduction in vital cells consistent with triggering HR. This was also seen in transgenic Desireé expressing *R3a* and *Pex147* was renamed *Avr3a*.



Co-infiltration of *N. benthamiana* with *A. tumefaciens* carrying a construct expressing *R3a,* and a strain expressing the truncated K-I *Avr3a* sequence resulted in confluent cell death. In contrast, co-expression of *R3a* with the truncated E-M *avr3a* sequence, or infiltrations of individual *A. tumefaciens* strains and other controls, failed to elicit visible cell death.

CONCLUSION:

Association genetics was used to identify the P. infestans Avr3a gene and show that its product is recognised in an R3a¬dependent manner in the host cytoplasm. Analysis of the Avr3a locus revealed unexpected conservation of synteny with the locus containing ATR1NdWsB in H. parasitica, supporting comparative genomics as an approach to investigate evolution of pathogenicity in oomycetes. The isolation of R3a and Avr3a represents an opportunity to investigate the earliest recognition events in a potato-P. infestans R-AVR interaction, and of subsequent signalling pathways leading to disease resistance. It also opens a door to studies of molecular mechanisms potentially underlying the biotrophic and necrotrophic phases of the P. infestans infection cycle.

ACKNOWLEDGEMENTS

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