A new molecular tool box for the detection of *Phytophthora* species threatening forests and other natural ecosystems

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Introduction

In recent years several new species of *Phytophthora* have been discovered. These pathogens damage important natural ecosystems and are damaging forests across the world. With a few notable exceptions, our understanding of the occurrence these species is based on traditional isolations and it is clear that PCR-based molecular diagnostics have great potential as tools to detect specific species or lineages of *Phytophthora*.

Aims & Approaches

- To develop a very sensitive multi-species diagnostic for Phytophthoras found on trees and other woody hosts.
- An alternative to ITS rDNA regions was sought to overcome difficulties in discriminating amongst some closely related taxa.
- Candidate target regions were identified as follows;
  - A fragment of the intergenic spacer (IGS) region (Fig. 1)
  - A fragment of the ras-related protein gene (Fig. 2)
  - Four different intergenic spacers in mitochondrial DNA (Fig. 3)
- Regions ranging from 300 to 950 bp were amplified and sequenced from 29 *Phytophthora* species including many important forest pathogens (*P. alni, P. cambivora, P. cinnamomi, P. citricola, P. europaea, P. inundata, P. lateralis, P. nemorosa, P. pseudosyringae, P. psychrophila, P. quercina, P. ramorum, P. ilicis*).

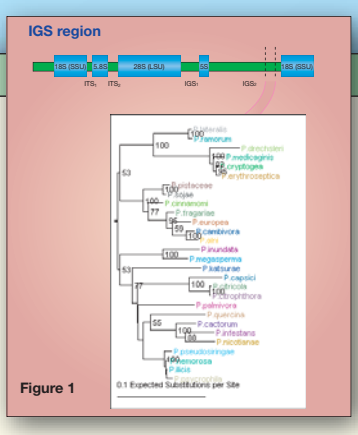

Figure 1

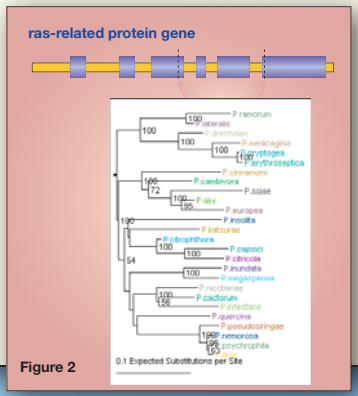

Figure 2

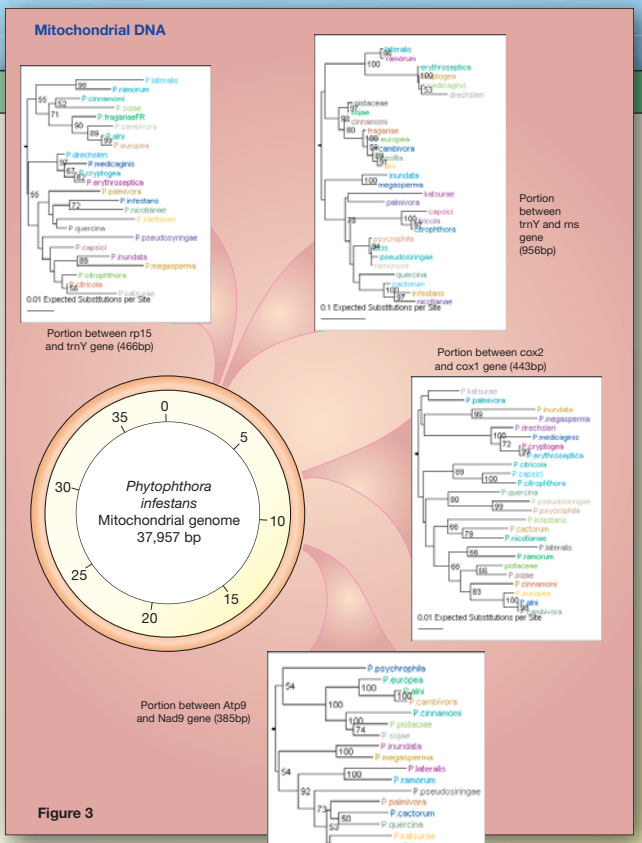

Figure 3

Results & Discussion

Alignment of sequences showed very variable as well as conserved regions suitable for the design of primers with different levels of specificity (Fig. 4)

The IGS fragment (approximately 450 bp) was the least variable region analysed but discrimination amongst some important species, not distinguishable using ITS sequences was possible (Fig. 1) . Furthermore, these sequences should facilitate the amplification and sequencing of larger, more variable, regions of the IGS. The IGS region has great potential since it is multicopy and its length provides considerable scope for primer development.

The ras-related protein gene (approximately 450 bp) comprised conserved coding regions and highly variable non-coding regions (Fig. 2). This gene has never been utilised in species identification. Despite being single-copy, it was easily amplified from most species and yielded strong PCR bands. Coding and non-coding regions could be utilised to design primers with appropriate levels of specificity.

As expected, across all four mitochondrial DNA fragments non coding regions were much more variable than coding regions (Fig. 4). All the mitochondrial DNA fragments have great potential for the development of specific molecular markers. However the fragment between the trnY and rns genes was the most attractive. This fragment was very variable with a total length ranging from 330 to 950 bp depending on the species analysed. The non-coding region was sufficiently variable for primers specific to almost all species, whereas the more conserved flanking coding regions were appropriate for the design of *Phytophthora*-genus specific primers. This location is thus suited to nested PCR in which amplified products from genus-specific primers could be used as a common template for a second round reactions with species-specific primers. A difficulty with mitochondrial regions is that they are A/ T rich (Fig. 4), making the design of primers quite difficult.

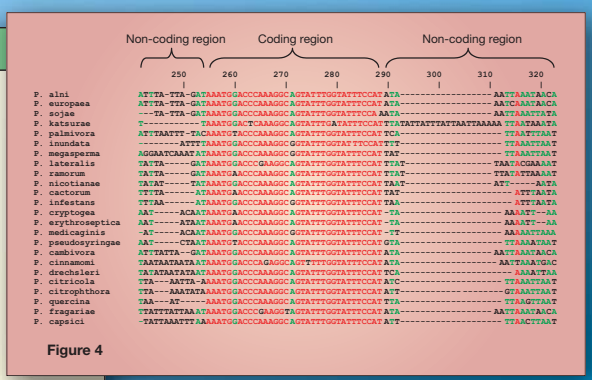

Figure 4

Conclusions

Six genomic regions were amplified and sequenced and their potential use for diagnostics assessed. Key studies on the intra-specific variation and primer specificity remain. However the project has already yielded an enormous dataset for the identification, detection and study of the molecular evolution of *Phytophthora* species.