

# RELATIONSHIPS WITHIN AND BETWEEN PHYTOPHTHORA AND OTHER OOMYCETES AS REVEALED BY TWO TYPES OF MOLECULAR MARKERS

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## INTRODUCTION

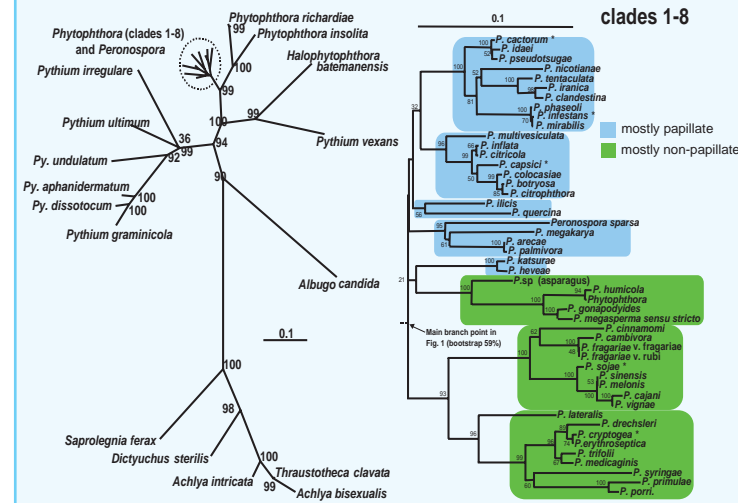
The genus *Phytophthora* in the Oomycota comprises ~60 species that are among the most important plant pathogens worldwide. They cause economically important rots and blights of a wide range of hosts but their identification and isolation are difficult, requiring much specialised expertise. This poster describes how DNA sequences and RFLP of part of the genomic rRNA gene repeat (rDNA) delineate species and relationships among them and with other Oomycota. Structure within species has been resolved by AFLP

## METHODS

The sequences of Internally Transcribed Spacers 1 and 2 (ITS1, ITS2) and the 5.8S gene which they flank (collectively - ITS), obtained by universal PCR primers, have been used to determine the affinities of *Phytophthora* spp. to one another and other Oomycota. ITS-RFLP coupled with computer-aided cluster analysis has been used to provide a rapid and reliable means of species identification. Linked to ITS studies, intraspecific diversity has been examined by Amplified Fragment Length Polymorphism (AFLP) analysis.

## RESULTS

Phylogenetic analysis demonstrated the evolutionary distance between Saprolegniales and Peronosporales (*Phytophthora*, *Peronospora* and *Pythium*). *Phytophthora*, unlike *Pythium*, was monophyletic with all key species forming a single clade with *Peronospora* and other downy mildews. It subdivided broadly into papillate and non-papillate groups.



Phylogeny of *Phytophthora* and other genera based on the alignment of sequences of 5.8S and ITS2. ITS1 was too variable for this scale of resolution. The blister rust (*A. candida*) did not cluster with *Phytophthora* or downy mildews

More detailed phylogeny of *Phytophthora* based on alignment of sequences of ITS1, 5.8S gene and ITS2. All but three species grouped in clades 1-8.

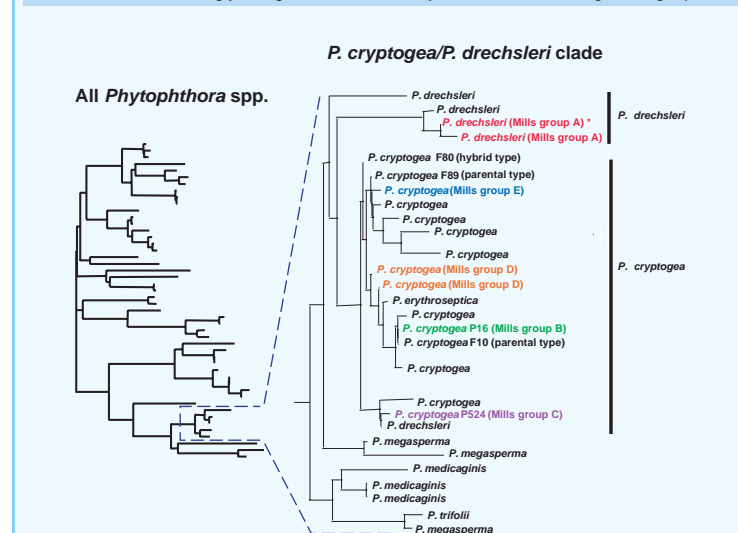
ITS-RFLP was sensitive enough to be able to discriminate the majority of *Phytophthora* species. Other tools are needed to separate closely related taxa such as *P. fragariae* varieties and *P. cryptogea* and *P. drechsleri*. Gels (A) were standardised (B), combined (C) and incorporated into a computer database GelCompar



GelCompar was used to compare the combined digest patterns to those in the database (>350). Isolates with no database match are sequenced and entered into the sequence database. The standard digest patterns of nearly all economically important species are given below and on the WEB (<http://www.cabi.org/BIOSCIENCE/phytophthora/index.htm>)

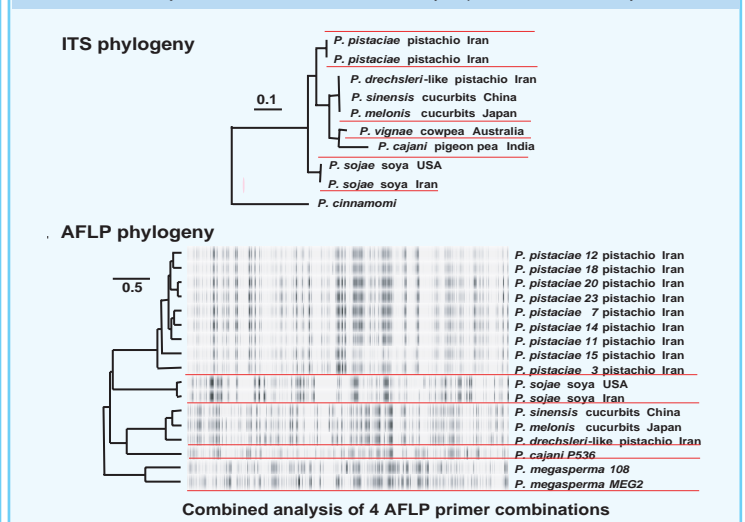


ITS can provide some resolution of fine structure within clades as shown below for *P. cryptogea* and *P. drechsleri*, which are difficult to distinguish from one another. Some isolates have been wrongly assigned to the clade by classical criteria e.g. *P. megasperma*.



Phylogenies based on sequence of ITS1-5.8S-ITS2 (\* isozyme grouping of Mills et al., 1991)

AFLP reveals markers dispersed throughout the genome and can separate taxa. In the example below it has been used to distinguish the taxa within a sub-clade of the genus that contains *P. sojae*, the cause of the economically important root rot of soybean.



In the example above, the affinities of isolates causing gummosis of pistachio in Iran have been re-examined by ITS and AFLP. Isolates originally assigned to *P. megasperma* and *P. drechsleri* have been described as *P. pistaciae* sp. nov. and *P. melonis* respectively. The latter also subsumes *P. sinensis*. Their relationships with the rest of the clade are shown. There was good concordance between ITS and AFLP trees.

## CONCLUSIONS

- ITS-PCR followed by sequencing or ITS-RFLP offers a powerful tool for the rapid identification of *Phytophthora*
- An expanding international database offers sufficient resolution to determine affinities of newly discovered species
- Phytophthora* is 'natural' genus within the Peronosporales within which its closest relatives are the downy mildews: *Peronospora*, *Pseudoperonospora*, *Bremia* and *Plasmopara* (results for the last three not shown).
- As with any other taxonomic character, sole reliance on ITS analysis is not recommended. It is most powerful when used in combination with other molecular or classical methods of analysis.
- AFLP analysis offers the finest scale of intraspecific resolution though it is costly and time-consuming.
- The ITS sequence database has been the basis of some highly sensitive and specific PCR diagnostics for phytophthora diseases, detecting the pathogen in plants, soil and water.

## REFERENCES

- Cooke et al. (2000) A molecular phylogeny of *Phytophthora* and related Oomycetes. *Fungal Genetics and Biology* 30: 17-32.
- Mills et al. (1991) Taxonomic structure of *Phytophthora cryptogea* and *P. drechsleri* on isozyme and mitochondrial DNA analysis. *Mycological Research* 95: 31-48.