Detection of *Phytophthora* in plants by the Polymerase Chain Reaction

J.M. DUNCAN[1], D.C. GUY[1], I LACOURT[1], P.J.M. BONANTS[2], J.A. MURPHY[3] and D.E.L. COOKE[1]

[1] Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland
[2] IPO-DLO, Wageningen, The Netherlands
[3] MRS Ltd , Invergowrie, Dundee DD2 5DA, Scotland

Introduction

Many important phytophthora diseases of horticultural and perennial crops, such as raspberry root rot (see right) are spread through planting vegetatively propagated stocks cryptically infected with the pathogen. Highly specific, sensitive and rapid tests for planting material are needed to prevent such spread. Existing methods of detection are limited either by lack of sensitivity or specificity or by being too time-consuming. The polymerase chain reaction (PCR) offers speed and specificity and has been used extensively for the detection of plant pathogens. This poster demonstrates the potential of the polymerase chain reaction for detection of *Phytophthora* species in plant material.

Requirements

A nested PCR, based on the sequences within the ribosomal gene repeat (rDNA), applicable to all *Phytophthora* spp. in plants, water and, eventually, soil. To date, tests have been developed only for species of importance to UK horticulture: *P. fragariae* (var. *fragariae*, red stele of strawberry; var *rubi*, raspberry root rot); *P. cambivora, P. cinnamomi* and *P. cactorum* (fruit crops and woody ornamentals) and *P. nicotianae and P. cryptogea* (glasshouse crops). However, the approach is generally applicable to all species.

Primers and Nested PCR

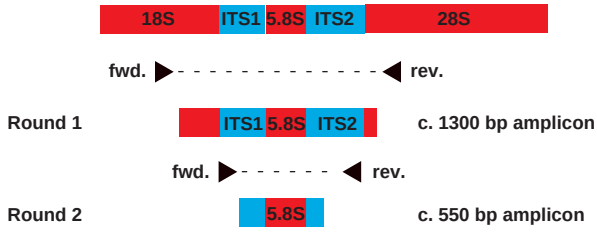
Primer DC6

This primer was designed after alignment of published DNA sequences of 18S subunits of rDNA of Oomycota, fungi and plants.

Alternaria	.CTTAGAGAGACTATCAAC---TCAAGTTGA.
Chlamydomonas	.CTTAGAGGGACTATTGGCG--TTTAGCCAA.
Achlya	.CTTAGAGGGACTTTCAGTGACT--AACTGA.
Lagenidium	.CTTAGAGGGACTTTTGGGTAATCAAACCAA. DC6
Phytophthora	.CTTAGAGGGACTTTTGGGTAATCAAACCAA.
Rice	.CTTAGAGGGACTATGGCCG--CTTAGGCCA.
Soybean	.CTTAGAGGGACTATGGCCG--TTTAGGCCA.

In combination with the universal primer ITS4, it produced an amplicon from all *Phytophthora* spp. and related genera, such as *Pythium* and the downy mildews, without amplification of plant DNA. In each case, the amplicon contained parts of the 18S and 28S subunits and all of the ITS1 and ITS2 regions and 5.8S subunit (see below).

Nested PCR



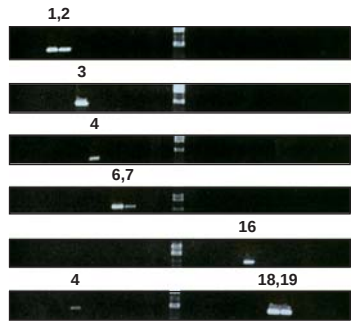
Specific primers

Specific primers for individual *Phytophthora* species were obtained by aligning rDNA of >50 species. A combination of six forward primers in ITS1 and four reverse in ITS2 gave the required specificity for the species listed above. In each case, the primer combination produced an amplicon which contained parts of ITS1 and ITS2 and all of the 5.8S subunit, and therefore could be used in nested PCR after amplification with DC6 and ITS4.

With a database of over 200 sequences from > 50 species, it should be possible to extend this nested PCR approach to any species of interest. Where very closely related species are very similar in their ITS sequences (see next panel), PCR diagnostics may only be possible for that group.

Specificty, Sensitivity and Results

Specificity



Primer combination for

P. fragariae

P. cambivora

P. cinnamomi

P. cryptogea

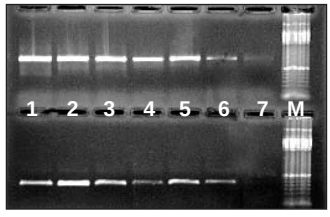
P. nicotianae

P. cactorum/P. idaei

Lanes 1-19: DNA from [1]*P. fragariae* v. *fragariae*, [2]*P. fragariae* v. *rubi*, [3]*P. cambivora*, [4]*P. cinnamomi*, [5]*P. sojae*, [6]*P. cryptogea*. [7]*P. drechsleri*, [8]*P. gonapodyides*, [9]*P. megasperma*, [10]*P. citricola*, [11]*P. capsici*, [12]*P. citrophthora*, [13]*P. palmivora*, [14]*P. megakarya*, [15]*P. ilicis*, [16]*P. nicotianae*, [17]*P. infestans*, [18]*P. idaei*, [19]*P. cactorum*.

Certain species such as *P. cactorum* and *P. idaei*, and *P. cryptogea, P. drechsleri* and *P. erythroseptica* are so similar at the ITS level that they cannot be distinguished from one another.

Sensitivity



Primer combination

DC6 & ITS4

DC1 & DC5
(*P. fragariae-specific*)

Lanes 1-7: DNA from mixtures of healthy and diseased strawberry roots infected with *P.fragariae*: 1. 100% diseased (d); 2. 20% d + 80% healthy (h); 3. 10% d + 90% h; 4. 5% d + 95% h; 5. 1% d + 99% h; 6. 0.1% d + 99.9% h; 7. 100% healthy; M - marker lane.

Simple extraction procedures yield DNA of suitable quality for PCR from 'difficult' substrates such as woody raspberry roots and although most work has been with *P. fragariae* on strawberry and raspberry, PCR works well for all the species listed in the introduction. It can also detect very small numbers of zoospores (~ 5) in drainage water.

Conclusions

Nested PCR based on rDNA sequences is a sensitive, specific and rapid method for detecting *Phytophthora* spp. in plant material. It can also detect very small numbers of zoospores in water (>5) and might be applicable for testing soil for contamination. The work presented above is the basis of an European Union SMT programme, the aim of which is to develop standardised tests which will be used throughout Europe to ensure propagation stocks of strawberry are free from red stele (*P. fragariae* var. *fragariae*). Partners are SCRI (UK), IPO-DLO (NL), BBA (D) and SLU Se).