

Using Single Strand Conformational Polymorphisms (SSCP) to Identify *Phytophthora* Species in Oregon Forests Affected by Sudden Oak Death¹

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Introduction

Phytophthora species are abundant in streams, widespread in soils and occasionally found in diseased plants in the tanoak forests of southwestern Oregon. It is time-consuming and expensive to identify hundreds of isolates to species using morphology or internal transcribed spacer (ITS) sequencing. We modified a published *Phytophthora* single strand conformation polymorphism (SSCP) protocol (Kong and others 2003) to use fluorescent-labeling chemistry and an additional marker locus to allow quantitative matching of unknown isolates.

Methods

The ITS1 region of rDNA was amplified with primers ITS6 and ITS7 (Cooke and others 2000) labeled with fluorescent HEX and FAM, respectively, yielding approximately a 300-bp product. The mitochondrial COX gene spacer region (Martin and Tooley 2003) was amplified with primers FMPh8 and FMPh10 labeled with fluorescent HEX and FAM, respectively, yielding approximately a 500-bp product. Amplified products were mixed with formamide and ROX 500 marker, heated to 95 °C for 3 minutes and cooled in ice for 5 minutes. Samples were run on an ABI 3100 Capillary Sequencer with a 36-cm array and 4 percent GeneScan polymer, with 10 percent glycerol, and 1X TBE, at 25°C. Fluorescence was analyzed with GeneScan 3.7 and electrophoretic mobility reported as scan number.

Results

Electropherograms generated by GeneScan typically revealed one peak for the forward strand and one peak for the reverse strand in both ITS1 and COX gene spacer regions. All reference

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isolates were well separated, except when comparing the closely related *P. cambivora* and *P. europaea*. Repeat analysis of references showed good repeatability.

We drew a blind sample of 54 unknowns from a larger collection of isolates from streams, soil and plants, and ran a preliminary test with 16 reference *Phytophthora* species, chosen for their perceived relevance to the sampling region. The sixteen references consisted of *P. cambivora*, *P. citricola*, *P. citrophthora*, *P. europaea*, *P. fragariae*, *P. gonapodyides*, *P. hibernalis*, *P. ilicis*, *P. lateralis*, *P. megasperma*, *P. nemorosa*, *P. pseudosyringae*, *P. psychrophila*, *P. ramorum*, *P. syringae* and *P. taxon 'Pg chlamydo.'* SSCP separated the 54 unknowns into 11 distinct groups. Five isolates matched taxon 'Pg chlamydo,' six isolates matched *P. nemorosa*, 22 isolates matched *P. gonapodyides*, and one isolate matched *P. ramorum*. The remaining seven unmatched groups suggest a large diversity of *Phytophthora* species in natural environments. Four isolates from one unique group have been characterized by ITS sequence and culture morphology, and appear to be a new species of *Phytophthora*. Several of the unknown isolates from stream samples were identified as *P. nemorosa*, previously known only from tanoak cankers in our area.

References

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