AFLP Analysis of Single Zoospore Isolates of *Phytophthora ramorum*

Christopher Fung, Kelly Ivors, Matteo Garbelotto
Department of ESPM-ES, University of California, Berkeley, CA, 94720

Introduction

Phytophthora ramorum, the causal agent of Sudden Oak Death, is assumed to reproduce asexually through a variety of scenarios. One possibility is the production and spread of highly motile spores called zoospores (Fig. 1). Produced mitotically in the sporangia, zoospores are presumed to be genetically identical. Thus, DNA fingerprinting of zoospores generated from the same parent isolate should yield identical fingerprint profiles.

Objective

To determine the amount, if any, of genetic variation between single zoospore isolates derived from the same parent isolate.

Amplified Fragment Length Polymorphism

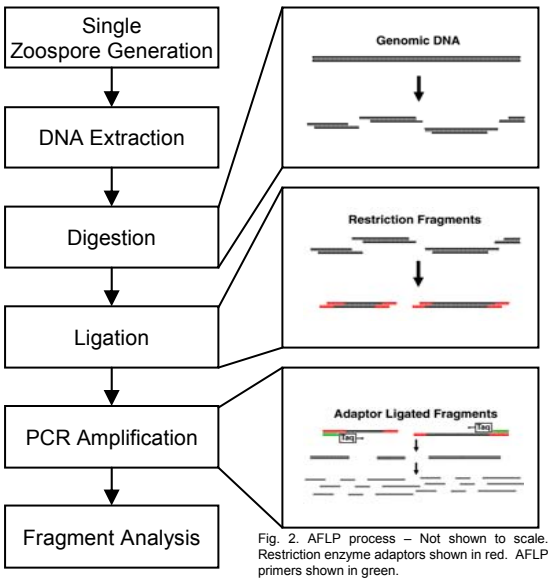

Fig. 2. AFLP process – Not shown to scale. Restriction enzyme adaptors shown in red. AFLP primers shown in green.

Materials and Methods

Single Zoospores

- 50 total isolates
- 5 parent isolates (Table 1.)
- 10 single zoospores generated from each parent

Fig. 1. Zoospores emerging from sporangia.

Parent Isolate	Host Species	Origin County
PR-2	*Lithocarpus densiflora* (Tan Oak)	Marin, CA
PR-5	*Lithocarpus densiflora* (Tan Oak)	Marin, CA
PR-153	*Umbellularia californica* (Bay laurel)	Solano, CA
PR-1	*Quercus agrifolia* (Coast live oak)	Marin, CA
PR-62	*Quercus agrifolia* (Coast live oak)	Sonoma, CA

Table 1. Origin of parent isolates used in experiment. Ten single zoospore isolates, numbered SZ.1 through SZ.10 were generated from each parent.

AFLP Protocol (Fig. 2)

- Adapted from Vos *et al.* (1)
- Restriction enzymes used:
  - *Mse*I and *Eco*R1
- Two rounds of PCR used; preamplification and selective amplification

Primer Sequences

- *Eco*RI (E00) (5'-GACTGCGTACCAATTC-3')
- *Mse*I (M00) (5'-GATGAGTCCTGAGTAA-3')
- EBTC (5'-A-E00-TC-3')
- EBAC (5'-A-E00-AC-3')
- EGG (5'-E00-GG-3')
- EGC (5'-E00-GC-3')
- MAG (5'-M00-AG-3')
- MAC (5'-M00-AC-3')
- MCC (5'-M00-CC-3')
- *Eco*R1 based primers labeled with FAM at 5' end
- Sequence shown in red indicates homology to ligase adaptor sequence

Results

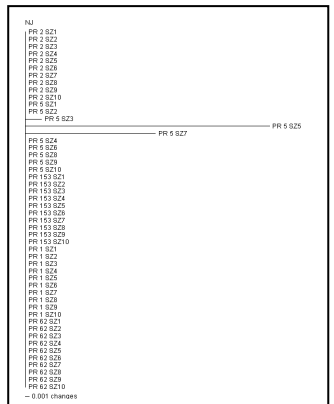

Fig. 3. Phylogram indicating the degree of genetic variation between all fifty single zoospores isolates.

- 231 total fragments, from 4 primer combinations analyzed
- 23 informative fragments, all from one parent: PR-5
- 4 AFLP genotypes identified
  - Clone group
  - PR-5 SZ.3 (1 of 23 informative fragments)
  - PR-5 SZ.5 (14 of 23)
  - PR-5 SZ.7 (8 of 23)

Discussion

Of the 23 informative fragments, only one was actually a missing fragment (PR-5 SZ.5). This indicates that unique fragments in the fingerprint profiles of the three variable isolates may have been due to non *P. ramorum* DNA. However, due to limitations of the AFLP technique, we were unable to conclusively determine either the presence or absence of foreign DNA. These limitations include both poor amplification of specific fragments and inability to score fragments below 60 bp and above 500 bp.

Preliminary Conclusions

- No variation between single zoospores, except among three single zoospores of one parent isolate (PR-5).
- Existence of all three variable genotypes in zoospore-set derived from one parent indicates an isolated anomaly.
- Continued investigation of PR-5 in progress.

References

1. P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, M. Zabeau, *Nucleic Acids Research* 23, 4407-4414 (1995).