What Can Availability of the *Phytophthora ramorum* Genome Do for Us?[1]

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Abstract

The complete genomes of *Phytophthora ramorum* and *P. sojae* have recently been sequenced. Of the 19,027 predicted genes in *P. sojae* and 15,743 gene models in *P. ramorum*, 9,768 are predicted to have the same function. These two genomes both revealed a rapid expansion and diversification of many protein families associated with plant infection including different classes of pathogen effectors. Two protein motifs (RxLR and dEER) are shared by the four known effectors in plant pathogenic oomycetes. Genome analyses identified a diverse superfamily of approximately 350 genes in *P. ramorum* that share these motifs. These have been termed avirulence homolog (Avh) genes. We were able to clone homologous Avh loci from the *P. ramorum* sister-taxa *P. lateralis* and *P. hibernalis.* Availability of the *P. ramorum* genome sequence has also resulted in several practical applications including identifying which genes are differentially expressed during different pathogen life-stages and during plant infection. The genome has been mined for molecular loci that can be used for pathogen identification and genotyping. Both the *P. ramorum* and *P. sojae* genomes have been used to select sequence loci for the construction of a phylogeny of the genus *Phytophthora*. The *P. ramorum* genome has also been helpful in finding simple sequence repeats and sequence loci that have been used to study the population structure and migration of clones. Availability of the *P. ramorum* and *P. sojae* genome sequences has already provided advances and a new understanding of *Phytophthora* biology. Many promising new discoveries are sure to follow.

Key words: *Phytophthora ramorum*, comparative genomics, genome.

Introduction

The complete genomes of *Phytophthora ramorum* and *P. sojae* were sequenced in 2004 (Tyler and others 2006). One obvious question arising is what contributions the availability of a genome sequence has for understanding the biology of *Phytophthora* spp. and the implications for management of sudden oak death (caused by *P. ramorum*) in the field. Simultaneous sequencing of two *Phytophthora* genomes allowed for rapid and direct comparison for similarities and differences between these two organisms.

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Phytophthora Genomes

To date five Oomycete genomes have or are being sequenced, of which four include Phytophthora species (table 1). *Phytophthora ramorum* and *P. sojae* were sequenced in 2004 at the Joint Genome Institute. The potato late blight pathogen *P. infestans* and the *Arabidopsis* downy mildew pathogen *Hyaloperonospora parasitica* were sequenced in 2006 and *P. capsici* is being sequenced.

Table 1—Background information on Oomycete genomes sequenced to date

Species	Genome size (Mb)	Coverage[1]	Sequencing center[2]
Phytophthora ramorum	65	7.7×	JGI
Phytophthora sojae	95	9×	JGI
Phytophthora infestans	240	8×	BI
Phytophthora capsici	65	2×/20×	JGI
Hyaloperonospora parasitica	75	8×	WUGSC

[1] 2× coverage based on Sanger sequencing and /20× coverage based on 454 sequencing.
[2] JGI= Joint Genome Institute (http://www.jgi.doe.gov/); BI =Broad Institute (http://www.broad.mit.edu/); WUGSC = Washington University, Genome Sequencing Center (accessible via http://phytophthora.vbi.vt.edu/).

Genome Information

Of the 19,027 predicted genes in *P. sojae* and 15,743 gene models in *P. ramorum*, 9,768 are predicted to have the same function. These two genomes both revealed a rapid expansion and diversification of many proteins families associated with plant infection including different classes of pathogen effectors.

Examples of Usage of Genome Data

Pathogen effectors can serve a virulence function on behalf of the pathogen or trigger a rapid defense response in resistant hosts. Two protein motifs (RxLR and dEER) are shared by the four known effectors in plant pathogenic oomycetes. Genome analyses identified a diverse superfamily of approximately 350 genes in *P. ramorum* that share these motifs (Tyler and others 2006). These have been termed a virulence homolog (Avh) genes. We have investigated the molecular evolution of one such group of six Avh genes. Microarray data suggests that four of these genes are expressed in isolate Pr-102 (Press and Grünwald, unpublished). We sequenced the full coding region (approximately 400 bp) and flanking noncoding regions of each gene in the three *P. ramorum* lineages (Goss and Grünwald, unpublished). The number of polymorphic sites within genes ranged from one to 12, suggesting different evolutionary pressures among genes. We have further been able to obtain the sequence of homologous Avh genes in the closely related *P. hibernalis* and *P. lateralis*.

Availability of the *P. ramorum* genome sequence has also resulted in several practical applications. For example, we are investigating which genes are differentially expressed during different life-stages (e.g., mycelium, zoospores) and

during plant infection using microarrays developed from the current genome annotation (Press and Grünwald, unpublished). Figure 1 shows transcripts of

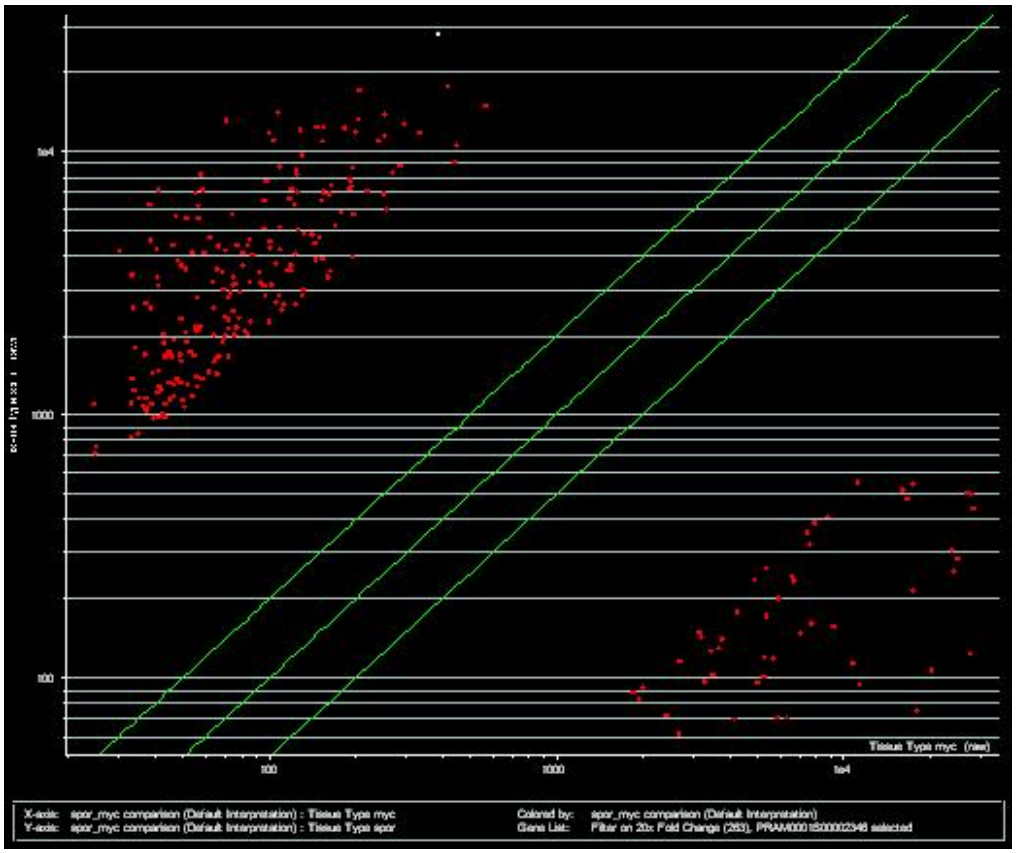

Figure 1—Red dots represent transcripts of *P. ramorum* with greater than 20-fold change in expression in mycelium as compared to sporangia (Press and Grünwald, unpublished) (x-axis: mycelium; y axis: sporangia).

P. ramorum that are more than 20-fold over or under-expressed in mycelial (x axis) versus sporangial (y axis) tissue.

The genome has been mined for molecular loci that can be used for pathogen identification and genotyping. The genomes have been helpful in selecting sequence loci that can be used for constructing a phylogeny of the genus *Phytophthora* (http://www.phytophthoradb.org/). Kang and colleagues have selected eight sequence loci that are phylogenetically informative to develop a multi-gene phylogeny of the genus *Phytophthora* (S. Kang, personal communication). Many studies have developed other molecular markers that can be used for pathogen identification using PCR (Hayden and Rizzo 2004; Kong, and others 2004; Tomlinson and others 2005; Hayden and others 2006; Hughes and others 2006a; Hughes and others 2006b; Ioos,and others 2006; Schena and others 2006; Tooley and others2006) or single nucleotide polymorphisms (Kroon and others 2004; Bilodeau and others 2006).

The *P. ramorum* genome has also been helpful in finding simple sequence repeats and sequence loci that have been used to study the population structure and migration

of clones (Prospero and others 2004, Ivors and others. 2006, Prospero and others in press). These studies have identified three distinct clonal lineages of *P. ramorum* in the U.S. that currently show no evidence of sexual recombination although both mating types have been found to co-occur in a few locations.

Outlook

Availability of the genome sequences has already provided advances and a new understanding of *Phytophthora* biology. Importantly, genome availability has provided timely tools for pathogen identification and genotyping. Availability of *Phytophthora* genomes has also provided leverage for obtaining additional resources for *Phytophthora* research. The Oomycete research community has been energized and a flurry of studies have already resulted from the availability of the genome sequences. Some of this research was recently highlighted in a special issue of Molecular Plant Microbe Interactions (Govers and Gijzen 2006, Jiang and others 2006, Krampis and others 2006, Lamour and others 2006, Meijer and Govers 2006, Meijer and others 2006; Tripathy and Tyler 2006, Zhang and others 2006). Many promising new discoveries are sure to follow.

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