The *Phytophthora* Species Known as "Pg chlamydo"[1]

Everett Hansen,[2] Paul Reeser,[2] and Wendy Sutton[2]

Abstract

Phytophthora taxon Pg chlamydo is perhaps the second most abundant *Phytophthora* species in the world, after *P. gonapodyides*, although it is commonly misidentified. Pg chlamydo is frequently encountered in streams and rivers in western North America, Argentina, China, and Europe. It has occasionally been recovered from forest soil and was once isolated from a bole canker on a tanoak tree; it was pathogenic to tanoak in artificial inoculation.

Pg chlamydo resembles *P. gonapodyides* in culture, and is related to that species. It is apparently sterile, not itself forming sexual structures even when paired with tester isolates of known mating type. Sporangia are non-descript nonpapillate, similar to other species in the *P. megasperma/P. gonapodyides* ITS clade. It is distinguished from *P. gonapodyides* by the formation of chlamydospores in culture. It has also been misidentified as *P. lateralis*, *P. drechsleri or P. cryptogea*, although the latter species are heterothallic, and readily distinguished with a mating test.

ITS-DNA sequences of isolates from Oregon, California, Argentina, and France were identical, but at least three mitochondrial genotypes were distinguished.

Introduction

Phytophthora taxon "Pg chlamydo" is perhaps the second most abundant *Phytophthora* species in the world, after *P. gonapodyides*, although it is commonly misidentified. It resembles *P. gonapodyides* in culture, but is distinguished by the formation of chlamydospores and chains of hyphal swellings. Like P. *gonapodyides*, it is a sterile species, but has also been misidentified as *P. lateralis*, *P. drechsleri or P. cryptogea*. "Pg chlamydo" is frequently encountered in streams and rivers in western North America, Argentina, and Europe. It has occasionally been recovered from forest soil and was once isolated from a bole canker on a tanoak tree; it is occasionally encountered as a foliar pathogen of woody plants in ornamental nurseries (Blomquist unpublished). It was associated with root rot of Port-Orford-cedar in German nurseries, where it was initially misidentified as *P. lateralis* (Hansen and others 1999), and with root rot and stem cankers of *Abies* species in nurseries and Christmas tree plantations, where it was misidentified as *P. drechsleri* (Brasier and others 1993).

Objectives

1. To raise general awareness of this common but poorly understood *Phytophthora*.

---

[1] A version of this paper was presented at the Fourth Meeting of IUFRO Working Party S07.02.09, Phytophthoras in Forests and Natural Ecosystems, August 26-31, 2007, Monterey, California.

[2] Department of Botany and Plant Pathology, Oregon State University, Corvallis OR 97331, USA. Corresponding author: hansene@science.oregonstste.edu.

2. To explore the population genetics of this sexually sterile and very widespread species.

Methods

Isolates recovered from water, soil and plant tissues originating from Europe (France and Germany), Argentina, and western United States (California and Oregon) were compared. All isolates from Argentina were baited from different locations on a single stream near Corcovado. Water isolates from western USA were all from different streams in SW Oregon and northern California.

Isolates were grown up on CMA and DNA was extracted from colonized agar plugs using a CTAB buffer protocol. DNA was PCR amplified with each of the five primer sets listed below (Table 1) and sequenced. These sequences were then edited and aligned.

Table 1—DNA regions analyzed.

Gene or DNA Region	Primers	Length Amplified	Citation
ITS rDNA	ITS 4 and ITS5	~820bp	White,T.J,.and others 1990
Mitochondrial COX spacer	FMPH8 and FMPH10	~340bp	Martin,F.N. and Tooley,P.W. 2003
60S ribosomal protein L10	60SL10F and 60SL10R	~420bp	http://www.phytophthoradb.org/
B tubuln	BTubF1 and BTubR1	~990bp	Kroon, L.P.N.M., and others 2004
Elongation Factor	EF1AF and EF1AR	~865bp	Kroon, L.P.N.M., and others 2004

Morphology

Morphology and growth were similar for all isolates examined regardless of source.

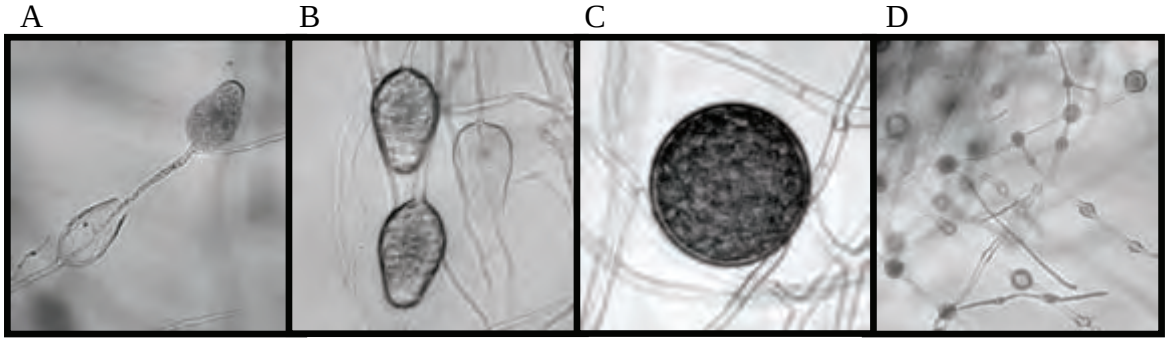

Figure 1—Sporangia of “Pg chlamydo” are non-papillate and proliferate both internally and externally (A and B). Chlamydospores (C) and hyphal swellings (D) characteristic of “Pg chlamydo”. Photos C. Delatour

Population Structure

Although isolates identified as “Pg chlamydo” are monophyletic within ITS clade 6, they exhibit remarkable intra-specific molecular variation in all genetic regions examined. “Double peaks,” commonly interpreted as evidence for past hybridization events, are unusually common in the DNA sequences. Only the mitochondrial COX spacer region lacked double peaks.

All isolates shared a basic ITS DNA sequence, but four ITS groups were distinguished based on the presence or absence of double peaks at four loci.
The mitochondrial COX spacer DNA region also had four genotypes among the 24 isolates fully characterized, although two genotypes were represented by single isolates. The 60S L 10 gene (RL10) encodes a ribosomal protein. Most isolates exhibited double peaks at 4-5 loci. Four related genotypes were identified among the isolates.
Elongation factor and β-tubulin gene sequences have not yet been not fully aligned, but many isolates exhibit double peaks at multiple loci.

Multilocus DNA Phenotype

With four genotypes recognized within each of the three DNA sequences fully analyzed, a total of 64 multi-locus genotypes are possible. Only five of these were identified in the 24 isolates fully characterized (Table 2).

Five isolates formed multi-locus Group A. There were no double peaks scored for Group A in any of the three DNA regions. These isolates were from streams and soil in Oregon, and water in France.

All of the isolates from Argentina were identical, and formed multi-locus Group B, distinguished by double peaks at four RL 10 loci, and a single ITS locus.
Multi-locus Group E had a distinctive COX spacer sequence and unique patterns of double peaks in the RL10 and ITS sequences. The ITS variation within Group E resulted from differences in relative peak strengths at the same double peak loci.
The remaining multi-locus Groups, Group C and Group D, were represented by single isolates.

Conclusions

Phytophthora isolates identified as P.sp.”Pg chlamydo” from Europe, North America, and Argentina are indistinguishable morphologically and closely related phylogenetically.

Isolates could be grouped in three main multi-locus DNA clades. Isolates from Oregon, California, and Europe were mixed in two clades, and all isolates from Argentina formed the third main clade.

Isolates in clade A (Oregon and France) exhibited no double peaks in the sequences analyzed. The other clades were distinguished by patterns of multiple double peaks.
P.sp.”Pg chlamydo” evidently has a complex evolutionary history.

Table 2—Groupings according to DNA alignments of three DNA regions.

Isolate	Source	Substrate	Collector	FMPH GROUP	60SL10 GROUP	ITS GROUP	MULTI-LOCUS GROUP
9364	Oregon	Tanoak canker	Hansen lab	I	c	2	A
33-2-2-0603	Oregon	Soil	Hansen lab	I	c	2	A
Haye3b	Europe	Water	Hansen & Delatour	I	c	2	A
WA42.2-062204	Oregon	Water	Hansen lab	I	c	2	A
WA49-031907	Oregon	Water	Hansen lab	I	c	2	A
AG27	Argentina	Water	Greslebin	I	d	4	B
AG29	Argentina	Water	Greslebin	I	d	4	B
AG30	Argentina	Water	Greslebin	I	d	4	B
AG48	Argentina	Water	Greslebin	I	d	4	B
AG50	Argentina	Water	Greslebin	I	d	4	B
WCK2.7.21	California	Water	Rizzo lab	II	e	1	C
WA46.3-100404	Oregon	Water	Hansen lab	III	d	2	D
107	Europe	POC soil	Hansen	IV	a	3.1	E1
62689	Europe	POC soil	Hansen	IV	a	3.1	E1
Haye3.1	Europe	Water	Hansen & Delatour	IV	a	3.1	E1
RIZZO-BUL 1-2.7.8	California	Water	Rizzo lab	IV	a	3.1	E1
WA20.1-041904	Oregon	Water	Hansen lab	IV	a	3.1	E1
WA38.1-083104	Oregon	Water	Hansen lab	IV	a	3.1	E1
WA39-100404	Oregon	Water	Hansen lab	IV	a	3.1	E1
WA6-011304	Oregon	Water	Hansen lab	IV	a	3.1	E1
H1	Europe	Water	Hansen & Delatour	IV	a	3.2	E2
WA48.2-070404	Oregon	Water	Hansen lab	IV	a	3.2	E2
RIZZO-RCK1.9.2	California	Water	Rizzo lab	IV	a	3.3	E3
WA5.1-072003	Oregon	Water	Hansen lab	IV	a	3.3	E3

Literature Cited

Hansen, E.M.; Streito, J.C.; Delatour, C. 1999. First confirmation of *Phytophthora lateralis* in Europe. Plant Disease 83:587.

Brasier, C.M.,Cooke, D.E.L.; Duncan, J.M.; Hansen, E.M. 2003. Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides-P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. Mycol. Res. 107(3): 277-290.

Brasier, C.M., Hamm, P.B.; Hansen, E.M.. 1993. Cultural characteristics, protein patterns and unusual mating behavior of *Phytophthora gonopodyides* isolates from Britain and North America. Mycol. Res 97:1287-1298.

Delatour, C. Atlas illustre de quelques Phytophthoras. Institut National de la Recherche Agronomique.

Kroon, L.P.N.M.; Bakker F.T.; van den Bosch, G.B.M.; Bonants, P.J.M.; Flier, W.G. 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genetics and Biology 41:766-782

Martin, F.N. and Tooley, P.W. 2003. Phylogenetic relationships among *Phytophthora* species genes. Mycologia 95: 269-284.

White, T.J.; Bruns, T.; Lee, S.; Taylor, J. 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: a guide to methods and applications (M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds): 315-322. Academic Press, Inc., New York.