

***Phytophthora siskiyouensis*, a New Species From Soil and Water in Southwest Oregon¹**

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Abstract

An unknown *Phytophthora* species was recovered from rhododendron and tanoak leaf baits used for monitoring streams and soils in Southwestern Oregon for the presence of *Phytophthora ramorum*. Isolates of this species yielded ITS-DNA sequences that differed substantially from other *Phytophthora* sequences in GenBank. Morphological features also differed from descriptions of known *Phytophthora* species. Based on the combination of unique morphology and unique ITS sequences, a new species is proposed. The new species, *Phytophthora siskiyouensis*, is homothallic, with globose to sub-globose oogonia, which may be terminal, sessile or lateral-intercalary. Antheridia are capitate and mostly paragynous, but sometimes amphigynous. Oospores are mostly aplerotic. Sporangia are ovoid to reniform, with apical, sub-apical, or lateral semi-papillae (occasionally more than one). Sporangia are terminal, sub-terminal, or occasionally intercalary on unbranched sporangiophores, with basal, sub-basal or lateral attachment. Sporangia are weakly deciduous, with variable length pedicels. This combination of characters clearly separates this taxon from other known *Phytophthora* species. *Phytophthora siskiyouensis* refers to the geographic region of origin.

Introduction

Phytophthora species are well known as pathogens of agricultural crops and of some forest species. Little is known of *Phytophthora* in natural settings. Ongoing surveys of *Phytophthora* species present in streams and soils in the sudden oak death epidemic regions of Oregon and California have revealed several undescribed *Phytophthora* species. In this paper we describe *Phytophthora siskiyouensis*, which is found in soil and water in the forested areas of coastal southwest Oregon.

Materials and methods

Isolates of *Phytophthora* were recovered from leaf or pear bait pieces plated in corn meal agar amended with 10 ppm natamycin (Delvocid[®]), 200 ppm Na-ampicillin, 10 ppm rifampicin, 25 ppm Benlate, and 25 ppm Hymexazol (CARPBH). Isolates were grown on corn meal agar amended with 20 ppm β -sitosterol (CMA β) to obtain mycelium for DNA extraction and storage as agar plugs in sterile de-ionized water.

Unknown isolates were analyzed by single strand conformational polymorphism (SSCP) of nuclear ribosomal internal transcribed spacer (ITS) and mitochondrial

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cytochrome oxidase (COX) spacer DNA fragments. ITS sequence analysis of isolates from one unique group indicated an undescribed species. Preliminary observations suggested the presence of shared morphological features consistent with a single new species.

For morphological study isolates were grown on clarified V8 agar amended with 20 ppm β -sitosterol (V8S). Oogonia and oospores were observed on 20 to 30 day-old cultures fixed and preserved in 3.7% formaldehyde. Sporangia were produced by transferring discs from margins of mycelium grown on V8S into natural stream water. Sporangia were fixed and preserved in 3.7% formalin, 10% acetic acid, 0.03% gelatin in 0.05M Na-K buffer (FA-PBG). Specimens were mounted in the fixative and viewed with brightfield illumination on a Zeiss standard microscope with a 63X objective, and measured with an eyepiece micrometer.

Results and discussion

Oogonia (average 25–30 μm) were formed abundantly on V8S in single culture and were usually globose to sub-globose, occasionally much elongated or with funnel shape tapering toward the stalk. Oogonia were terminal on long or short stalks (often bent or kinked), and were frequently sessile and occasionally unilaterally intercalary. Antheridia were predominately paragynous and capitate, with around 10 percent amphigynous. Antheridia were typically terminal, occasionally intercalary, and usually dichinous, attached anywhere on the oogonium. Oospores (average 23–26 μm) were globose to sub-globose, and usually aplerotic, sometimes markedly so in elongated oogonia. Hyphal swellings or chlamydospores were not observed.

Sporangia (average 46–70 μm length by 30–50 μm width) were formed sparsely in V8S agar and abundantly on agar culture pieces placed in stream water. Sporangia varied widely in shape, but were typically ovoid, reniform, or some misshapen variant of these. Overall length to breadth ratio was 1.5, ranging from 1.2 to 2.0 for individual isolates. Sporangia were scarcely semi-papillate with prominent thickening. Semi-papillae were applied apically, sub-apically or laterally, occasionally with two, and more rarely with three, papillae. Sporangioophores were simple, unbranched, short or long, attached basally, sub-basally or laterally to the sporangium. There was often a small hyphal swelling associated with the sporangioophore. Sporangia were typically terminal, but were often sub-terminal and occasionally intercalary, and weakly deciduous with variable pedicel length (average 28 μ , individual pedicels 0 μ to 133 μ).

P. siskiyouensis sporangia resemble those depicted for *Phytophthora quercina*, (Jung et al. 1999) except that they are slightly larger, semi-papillate, and weakly deciduous, and are formed singly on simple, unbranched sporiangioophores. Sexual structures also resemble *P. quercina*, except that antheridia may be paragynous or amphigynous and paragynous antheridia are attached anywhere on the oogonium. The oogonial stalk and arrangement of paragynous antheridia are similar to those described for *P. hedraindra* (de Cock and Levesque 2004).

Colonies on V8S were submerged with a faint radiate pattern and little or no aerial mycelium. Optimal temperature for growth of the nine isolates tested was 25 C. Radial growth rate averaged 7.5 mm/day at 25 C (individual isolates ranged from 6.2

to 8.5 mm/day at 25C). Incubation at 35 C was lethal, and cultures did not recover when removed to 20 C. Isolates which did not grow at 5 C or 30 C recovered when removed to 20 C.

DNA extracts from six *P. siskiyouensis* isolates were amplified with primers DC6 and ITS4. Amplified products were sequenced with DC6, ITS2, ITS3 and ITS4 as sequencing primers. The six *P. siskiyouensis* isolates were identical. Published sequences of other *Phytophthora* species were downloaded from GenBank and aligned using Clustal X v1.81. *P. siskiyouensis* clustered on a branch with *P. capsici* and *P. tropicalis*.

References

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