S10-1

Two Types of ITS in *Phellinus sulphurascens*, a Root Rot Fungus in British Columbia

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Phellinus sulphurascens Pilat, also known as the Douglas-fir form of *P. weirii* (Murr.) Gilb. (Lim et al. 2005), is an important pathogen causing laminated root rot in Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) and other conifers in western North America (Hansen and Goheen 2000; Thies and Sturrock 1995). In British Columbia (BC), the fungus occurs in the southernmost part of the province, coincident with the distribution of Douglas-fir. *P. sulphurascens* is especially prevalent in a variety of subzones within the Province's Coastal Douglas-fir (CDF), Coastal Western Hemlock (CWH), Interior Douglas-fir (IDF), and Interior Cedar Hemlock (ICH) biogeoclimatic zones. In infected stands, laminated root rot spreads mainly by growth and transfer of vegetative mycelia from infected roots contacting uninfected roots. *P. sulphurascens* causes root decay and frequently kills roots and whole trees (Hansen and Goheen 2000; Wallis and Reynolds 1965). After the death of infected trees, the fungus can live as a saprobe for 50 years or more in infected stumps and large roots (Hansen 1979a). Effective management of forest diseases, like laminated root rot, requires that we understand the population structure and life history of the causal agents. While the epidemiology of *P. sulphurascens* is relatively well described, little is known about the genetic composition of its population. A population study of *P. sulphurascens* in British Columbia (BC), Canada showed that two types of ITS sequences were present in the basidiospores and vegetative isolates; referred to as type 1 (731 bp) and type 2 (735 bp)

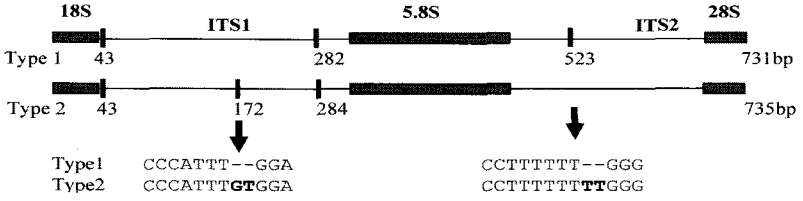

Fig. 1. Restriction map of ITS regions and sequences for the two different types found in *P. sulphurascens*. Sequences shown are for type 1 and type 2 single spore isolates derived from the parental heterokaryon of *P. sulphurascens* isolate PFC583. The GT insertion in type 2 occurs at position 173 while the TT deletion event in type 1 occurs at position 526.

(Fig. 1). RFLP marker with Bsl I can differentiate the two types of ITS sequences.

For all vegetative isolates of the 110 isolates of *P. sulphurascens* that were analyzed, some vegetative isolates were either type 1 or type 2, while others had both ITS sequences (Fig. 2). The occurrence of more than one ITS type has been found in other filamentous fungi belonging to Zygomycetes (Sanders et al. 1995), Ascomycetes (Aviram et al. 2004) and Basidiomycetes (Kauserud and Schumacher 2002; Ko and Jung 2002). Several explanations may account for the observe polymorphisms of rDNA (Ko and Jung 2002). Four nucleotide differences in two insertion/deletion sites suggest that neither ITS type 1 nor ITS type 2 were pseudogenes and that there was no hybridization event. Therefore, the most plausible explanation of two types of ITS in *P. sulphurascens* may be that independent mutations have given rise to two forms of nrDNA gene clusters within individual cells and that either one of the two ITS types were fixed later in separate gene clusters or nuclei.

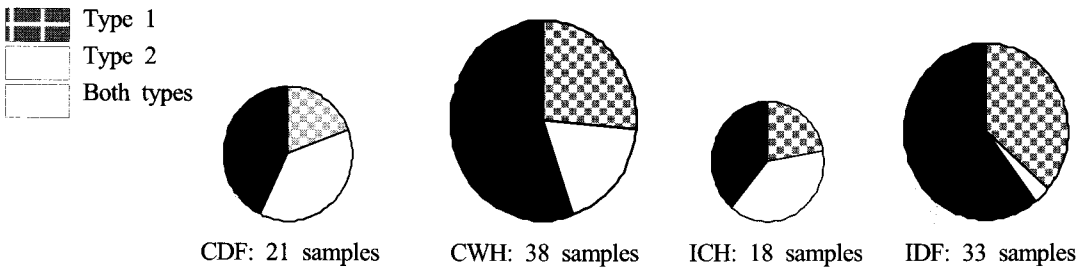

Fig. 2. The population structure of 110 *P. sulphurascens* strains with type 1 and type 2 ITS sequences collected from four biogeoclimatic zones in BC. CDF-Coastal Douglas-fir; CWH-Coastal Western Hemlock; ICH-Interior Cedar-Hemlock; IDF-Interior Douglas-fir.

Two models have been suggested to explain variation in ITS types; i) the diverse ITS variants may be distributed among different nuclei, ii) all nrDNA variants may be contained within each nucleus (Aviram et al. 2004; Kauserud and Schumacher 2003; Pawlowska and Taylor 2004; Selosse et al. 1996; Wu et al. 1998). In our work, a total of 101 single spore isolates were examined and each had only one of the two ITS types; the two types never occurred together. This suggests that the different ITS regions may be presented in different nuclei. Homokaryotic isolates of *P. sulphurascens* have multinucleate hyphae, with an average of about three nuclei per cell (Hansen 1979b). Although some binucleate spores have been reported (to ≈ 2%), multinucleate homokaryotic hyphae were derived from uninucleate basidiospores. Therefore, homokaryons carry all the same type of nuclei that contain only one type of ITS.

However, all single spore isolates had either type 1 or type 2. The two types never occurred together in single spore isolates. Single spore progenies derived from three different parental basidiocarps of *P. sulphurascens* (PFC541, PFC583, and WL12) contained both types of ITS. For PFC541 and PFC83 single-spore progenies, nine were type 1 and eight were type 2. For WL12 progenies, six were type

1 and nine were type 2 (Table 1). These basidiocarps, with both ITS types, show a simple Mendelian segregation pattern, in agreement with the inheritance of only one type of ITS in each basidiospore. A similar segregation pattern for different types of ITS regions has been observed in the diploid *Saccharomyces* (Petes and Botstein 1977), and in homobasidiomyceteous fungi; e.g. *Pleurotus cornucopiae* (Iracabal and Labarrère 1994), *Chondrosterum purpureum* (Ramsfield et al. 1996), and *Trichaptum abietinum* (Kauserud and Schumacher 2003). In our data, however, many parental isolates had only one ITS type and produced progenies with the same type of ITS. Progeny from a type 1 parental isolate had only a type 1 ITS sequence and progeny from a type 2 parental isolate had only a type 2 ITS sequence. For these strains, we cannot confirm that Mendelian segregation is occurring. These results suggest that for *P. sulphurascens* mating genes are not linked with the ITS regions. Little information is available on the mating system of *Phellinus* species and none is available on *P. sulphurascens*. Thus, further work is needed to confirm the relationship of ITS regions and mating type genes.

Table 1. Segregation of the ITS types among homokaryons derived from single spore (ss) isolates of parental basidiocarps originating in BC and Oregon.

Parental type[a]	ITS type 1	ITS type 2
MP8 (type 1)	ss3, ss5	
MP13 (type 1)	ss8	
MP16 (type 1)	ss1, ss2, ss3, ss9	
PFC603 (type 1)	ss1-ss20	
PFC547 (type 2)		ss1, ss2, ss3, ss4, ss5
PFC568 (type 2)		ss1-ss19
PFC583 (both)	ss3, ss6, ss9, ss11, ss12, ss14, ss15, ss17	ss2, ss4, ss5, ss7, ss8, ss10, ss16, ss18, ss20
PFC541 (both)	ss2, ss4, ss6, ss8, ss9, ss15, ss19, ss20	ss1, ss5, ss7, ss10, ss11, ss12, ss13, ss14, ss18
WL12 (both)	ss1, ss2, ss5, ss9, ss11, ss15	ss3, ss4, ss6, ss7, ss8, ss10, ss12, ss13, ss14, ss15

[a]MP-Mary's Peak, Corvallis Oregon, USA; PFC-Pacific Forestry Centre, Victoria, BC, Canada; WL12-Alex Fraser Research Forest of UBC, Williams Lake, BC, Canada.

Kauserud and Schumacher (2002) detected 20 variable sites in the ITS sequences (730 bp) of ten *Phellinus nigrolimitatus* (Rom.) Bourd. et Galz. single spore isolates. This species is phylogenetically close to *P. sulphurascens*. However, only two variable sites were detected in *P. sulphurascens* isolates from BC. This low ITS variation agrees with the overall low genetic variation that we have found for BC isolates using RAPD analysis with M13 primers, as well as in comparing sequence data for several genes (data not shown). The low ITS variation might also be the result of *P. sulphurascens* being a more recently derived species than *P. nigrolimitatus* (Wagner and Fischer 2002).

ITS-RFLP results generated information on the ITS polymorphism of *P. sulphurascens* in the four biogeoclimatic zones in BC where they most commonly occur. Both ITS types occurred in varying ratios over the four zones. Fifty-seven isolates had both types 1 and 2 ITS, while the others had only one type (Table 1). Although genetic differentiation of the ITS at the population level was not observed within BC's population of *P. sulphurascens*, it is likely that this species had two types of ITS when

it arrived in western North America millennia ago from its place of origin.

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