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Identification of groups within *Laetiporus sulphureus* in the United States based on RFLP analysis of the nuclear ribosomal DNA

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Abstract: One hundred sixteen collections of *Laetiporus sulphureus* from throughout the United States were analyzed for variation in the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA. The region was amplified using primers ITS 4 and ITS 5 in a polymerase chain reaction (PCR) and was the same size for all collections. The PCR products were digested with six restriction endonucleases and analysis of the resulting restriction fragment length polymorphisms (RFLP) identified seven *Laetiporus* restriction groups (LRG I-VII). Fruiting bodies within each LRG usually had the same color pore layer and occurred on similar host types (conifers vs. hardwoods) in similar positions on the host. Distinctive differences in morphological and ecological characteristics occurred between many of the LRGs. The RFLP pattern of LRG VII is suggestive of a heterozygous condition, composed of the pattern types occurring in LRGs I and VI. These three LRGs appear to be identical for the other characters examined and probably represent the true *L. sulphureus*. The apparent lack of individuals representing heterozygous combinations between the other four LRGs may indicate that these LRGs are reproductively isolated. One of these, LRG IV appears to be the white pored, ground-fruitiing species described as *Polyporus cincinnatus*, by Morgan in 1885. The combination *Laetiporus cincinnatus* is proposed for this species.

INTRODUCTION

Laetiporus sulphureus (Fr.) Murr. is a common, easily identified member of the Polyporaceae that occurs in most areas of the United States. It is pathogenic, causing a brown cubicle rot of roots, butts, and heartwood of living trees. In the eastern, central, and southwestern United States it occurs almost exclusively on hardwoods, especially *Quercus* spp., but is commonly found on conifers in the Rocky Mountains and Pacific Northwest (Gilbertson & Ryvarden, 1986). It is also a persistant saprophyte and can survive and fruit for many years from a colonized substrate (Hepting & Roth, 1950).

Although *L. sulphureus* is easily identified via its bright orange color, morphological diversity within the species has lead to the description of a number of varieties. *Laetiporus sulphureus* var. *sulphureus* is the type of the genus and has basidiocarps with a lemon yellow pore layer and occurs on standing trunks, stumps or downed logs. *Polyporus* sulphureus variety *semialbinus* Peck was described to accommodate specimens with white pore layers (Peck, 1906). This same form was more thoroughly described as variety *overholtsii* by Rosen (1927), who included white pored collections, with pink pelei fruiting from the ground in association with *Quercus* species. It also appears this form was described as *Polyporus cincinnatus* Morgan (1885). but this elevation to the species level has not been widley accepted (Overholts, 1953). Specimens occurring on conifers have also been considered as a distinct form and in Europe collections from *Picea* sp. were used to describe the species *L. montanus* Cerny (1989).

Relative uniformity of microscopic traits among varieties of *L. sulphureus* has hampered studies on their taxonomy. In addition, compatibility information is unavailable due to erratic basidiospore germination and the absence of clamp connections. However, with the advent of molecular biological techniques such as polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, studies of closely related taxa have become feasible. We intend to use these techniques in evaluating the relationships between populations of *L. sulphureus*. This paper represents the first step in this evaluation.

MATERIALS AND METHODS

Collections of *L. sulphureus* were obtained from many different hosts and locations in the United States and information on host, fruiting position and pore layer color were recorded. Complete collection information is available from the authors upon request. Tissue isolates were obtained from most of the collections by excising small pieces from the pileus context using aseptic technique, and placing them on 1.5 % malt extract (Difco, Detroit, Mich.) and 2% agar medium (MEA) in Petri plates. All isolates are on deposit in the Center for Forest Mycology Research (CFMR) culture collection maintained at the USDA Forest Products Laboratory in Madison, WI.

Template DNA for PCR was obtained via several different methods. Primarily, tissue isolates were grown on cellophane overlaying MEA, ground and diluted 1:100 for use in PCR using previously described techniques (Volk et al., 1996). In addition, scrapings of aerial mycelium from MEA cultures or small pieces of fruiting body pore layer and trama were treated in the same manner as mycelium from cellophane. Finally, for some collections DNA was extracted from mycelium grown in 10 ml of 2% malt extract 0.2% yeast extract (Difco) broth in 90mm Petri plates. The mycelium was filter harvested, freeze dried, ground in a sterile mortar and pestle with dry ice and then extracted for DNA using CTAB (Rogers et al., 1989). The DNA obtained was diluted 1:1000 for use in PCR.

Amplification of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA was accomplished using primers ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS-5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') in PCR. The reaction mixture and amplification conditions used were those described by Volk et al. (1996). Following amplification the products were digested with the following restriction endonucleases: *Hae* III. *Hind* III, *Hinf* I, *Msp* I, *Rsa* I, and *Hha* I (Promega, Madison, Wis.). Digests were run using the reaction buffer supplied with each enzyme, 5 µL of amplification product, 5 units of each enzyme and water to bring the final volume to 20 µL. The reactions were incubated overnight at 37°C and the resulting digestion products were separated on a 4% agarose gel. Fragment sizes were determined using the method of Volk et al. (1996).

RESULTS

Amplification products of the ITS region were obtained from 116 *L. sulphureus* collections. Five of these were amplified directly from the basidiocarps and the remainder from tissue culture mycelium. There appeared to be no difference in amplification product from the different sources of template DNA, so the data were combined for analysis. The size of the PCR product was about 650 bp for each of the samples. Digestion of the PCR products of the 116 samples with *Hae* III, Hind III, *Hinf* I, *Msp* I, *Rsa* I, and *Hha* I, yielded two, two, three, three, four and two different RFLP banding patterns, respectively (Fig. 1, Table 1). For each of the collections tested, collating the pattern types obtained from each enzyme resulted in one of seven unique combinations. Groups of collections exhibiting the same combinations of patterns were referred to as *Laetiporus* restriction groups (LRG) and were designated LRG I-LRGVII (Table 2). Twenty nine collections belonged to LRG I, 14 to LRG II, 11 to LRG III, 33 to LRG IV, 5 to LRG V, 1 to LRG VI and 23 to LRG VII. The relation of the LRGs to host, fruiting position, pore layer color, and geographic location are shown in Table 3.

DISCUSSION

All of the isolates in each LRG possessed the same pattern types for each of the six enzymes. Collections within each LRG had many other characters in common. All collections of LRGs I, III, VI and VII had a yellow pore layer and fruited from stumps, living and dead trunks, or logs. All collections of LRGs IV and V had white pore layers. LRGs II and V fruited from stumps, living and dead trunks, and logs. LRG II was the only group to show variation in pore color with collections from Louisiana having white pores and those from California having yellow. LRG IV was the only LRG to fruit on the soil from roots and was found in this position in 30 of 33 collections. The specimens of LRG IV appear to correspond to the description of *L. sulphureus* var. *semialbinus* (Overholts, 1953).

LRG III was the only group collected from conifers, and it appears to be restricted to these hosts. The other LRGs were collected most often on oak except for LRG II from California,

where it occurs most often on *Eucalyptus*. LRG IV occurs almost exclusively on oak, which is also the most frequently reported host for L. *sulphureus* var. *semialbinus*. LRGs I, V, VI and VII do not seem to be as closely associated with oak.

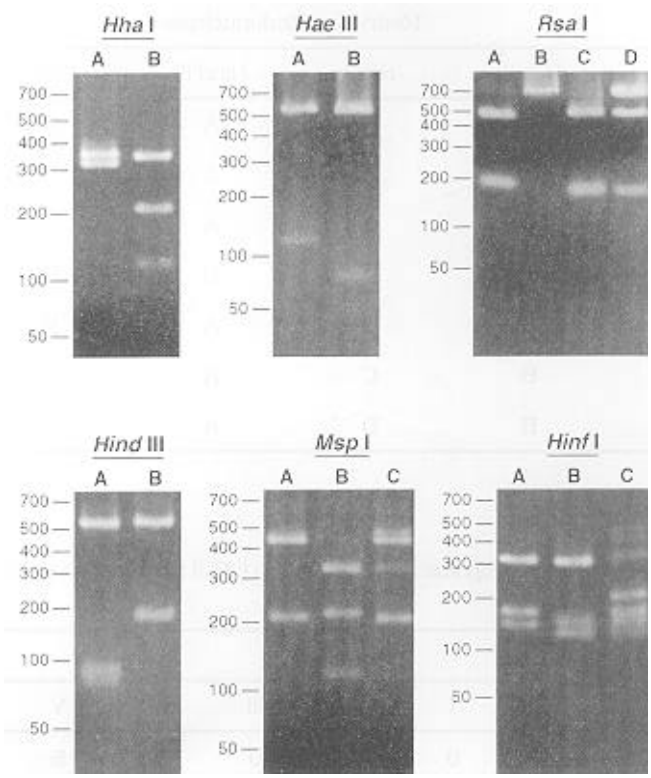

Fig. 1. Representative banding patterns obtained from the PCR amplified ITS region of 116 collections of *Laetiporus sulphureus* when digested with six restriction endonucleases.

Table 1. Approximate size in base pairs (bp) of the fragments obtained from the ITS region of the nuclear ribosomal DNA of 107 L. sulphureus isolates when digested with six restriction endonucleases.

	Pattern Type Designation			
enzyme	A	B	C	D
Hha I	375, 335	360, 210, 115	-	-
Hae III	550, 115	550, 60, 50	-	-
Rsa I	440, 180	660	460, 150	660, 460, 150
Hind III	530, 90, 80	560, 180	-	-
Msp I	450, 195	335, 210, 115	500, 450, 335, 195, 115	-
Hinf I	335, 170, 145	315, 140, 120	200, 170, 140, 120	-

Table 2. Designation of seven *Laetiporus* restriction groups (LRG) based on the RFLP pattern obtained from the ITS region of the nuclear rDNA when digested with six restriction endonucleases.

	Restriction Endonuclease					
LRG	*Hha* I	*Hae* III	*Rsa* I	*Hind* III	*Msp* I	*Hinf* I
I	B	B	B	A	A	A
II	A	A	B	A	B	B
III	A	A	A	A	B	C
IV	A	A	A	B	B	A
V	B	B	B	A	B	A
VI	B	B	C	A	B	A
VII	B	B	D	A	C	A

Table 3. Number of collections of each *Laetiporus* restriction group (LRG) that exhibit a specific morphological, distributional, or ecological characteristic.

		LRG						
		I	II	III	IV	V	VI	VII
pore layer color	white	0	4	0	29	5	0	0
	yellow	24	7	5	0	0	1	19
host	*Quercus sp.*	20	3	0	26	4	0	11
	other hardwood	9	10	0	4	1	1	7
	conifer	0	0	8	0	0	0	0
fruiting position	soil from roots	0	0	0	26	0	0	0
	trunks, logs and stumps	27	12	9	3	5	1	16
geographical location	northeastern	5	0	0	0	0	0	1
	southeastern	2	5	0	2	0	0	2
	midwestem	22	0	1	31	5	1	20
	northwestern	0	0	8	0	0	0	0
	southwestern	0	9	2	0	0	0	0

Complete distributions of the LRGs can not be determined definitely from this study. Due to the extensive sampling in the midwest more can be said about the LRGs occurring there than other areas. The absence of LRG II from this area may indicate that its range is restricted to warmer climates like those of Louisiana and California from which our collections originated. LRG III was originally believed to be found only in the west and northwest, until its collection in Michigan in 1997. This distribution may be closely tied to host preference, in this case old growth or mature conifers which are rare in east of the Rocky Mountains. LRG IV is one of the most common groups in the midwest, although it was collected rarely in other regions. This LRG was collected as far south as Louisaina and as far west as South Dakota. If this LRG is indeed the same as *L. sulphureus* var. *semialbinus* it is likely that it should also be common in the northeast, where it has been reported to be more common than the yellow pored form (Overholts, 1953). LRGs I and VII seem to be present throughout the eastern United States and LRG VI was only collected in Wisconsin.

While the RFLP patterns for most of the LRGs are distinctive, LRG VII may result from the combination of the patterns present in LRGs I and VI. These three groups have the same pattern when digested with enzymes *Hae* III, *Hha* I, *Hind* III, and *Hinf* I. Pattern D for *Rsa* I, present in LRG VII, has fragment sizes that could result from the combinations of patterns B and C which are found in LRG I and VI respectively. Pattern C for *Msp* I, present in LRG VII, likewise appears to be a combination of pattern A found in LRG I and pattern B found in LRG VI. Combined patterns such as these have been shown to be indicative of compatibilty between isolates with differing RFLP patterns in the intergenic spacer region of the rDNA for several species of *Armillaria* (Volk et al., 1996; Banik & Burdsall, 1998). The combined patterns found in LRG VII suggest a similar situation.

Reproductive isolation of the other LRGs may be inferred from the absence of RFLP patterns indicative of genetic exchange between them. This appears especially likely for LRG's I, IV and V which were collected extensively in Dane County, Wisconsin. Thirty four collections were made in Dane County but none possessed RFLP patterns suggesting they were interbreeding. In several instances two or three of the LRG's were collected in the same 1 hectare woods, with no evidence of combined patterns. Combined patterns involving LRG's II and III were also not detected, but the areas in which they occur were not extensively sampled.

The morphological and ecological data strongly associate LRG IV with the form described as *L. sulphureus* var. *semialbinus*. In addition the reproductive isolation indicated by the RFLP data strongly support this form as a distinct species. The oldest validly published name for the white-pored form occurring on roots or soil was described by Morgan (1885) as *Polyporus cincinnatus*. Therefore, for this species, we propose the new combination ***Laetiporus cincinnatus* (Morgan) Burdsall, Banik & Volk (comb. nov.),** (Basionym: *Polyporus cincinnatus* Morgan: J. Cincinnati Soc. of Nat. Hist. 8: 97, 1885). Also, we believe that LRGs I, VI, and VII probably all represent true *L. sulphureus*.

The taxonomic status of the LRGs II, III and V remain in question. The lack of RFLP patterns indicative of genetic combination between these groups coupled with consistent differences in pore color, host, and fruiting habit, suggests possible species status for several of these LRGs as well. Mating studies to determine the compatibility of these LRGs are currently underway in our laboratory. The results of those studies couple with further molecular biological and morphological data will lead to a clearer understanding of the relationships among the LRGs.

REFERENCES

Banik, M. T., & H. H. Burdsall, Jr. 1998. Assessment of compatibility in vitro among *Armillaria cepestipes*, *A. sinapina*, and North American biological species X and XI, using culture morphology and molecular biology. *Mycologia* 90: xxx-xxx.

Cerny, A. 1989. *Paraziticke Drevokazne Houby*. Prague. 99 p.

Gilbertson, R. L. & L. Ryvarden. 1986. *North American Polypores*. Vol. 1. *Abortiporus-Lindtneria*. Fungiflora, Oslo, Norway. 433 p.

Hepting, G. H., & E. R. Roth. 1950. The fruiting of heart rot fungi on felled trees. *J. Forestry* 48: 332-333.

Morgan, A. P. 1885. The mycologic flora of the Miami

Valley, Ohio. *Cincinnati Soc. Nat. Hist. Jour.* 8: 91-111.

Overholts, L. O. 1953. *The Polyporaceae of the United States, Alaska, and Canada.* University of Michigan Press. Ann Arbor, Mich. 466 p.

Peck, C. H. 1906. Report of the State Botanist, 1905. *N. Y. State Mus. Bul.* 105: 34.

Rogers, S. O., S. Rehner, C. Bledsoe, G. J. Mueller, & J. F. Ammirati. 1989. Extraction of DNA from Basidiomycetes for ribosomal DNA hybridizations. *Can. J. Bot.* 67: 1235-1243.

Rosen, H. R. 1927. A pink-colored form of *Polyporus sulphureus* and its probable relationship to root-rot of oaks. *Mycologia* 19: 191-196.

Volk, T. J., Burdsall, H. H., Jr., & M. T. Banik. 1996. *Armillaria nabsnona*, a new species from western North America. *Mycologia* 88: 484-491.