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Incompatibility groups among North American populations of *Laetiporus sulphureus* sensu lato

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Abstract: Mycelial interactions and allozyme analysis for glucose&phosphate isomerase activity were used to evaluate compatibility of pairings of single spore isolates (SSIs) within and between *Laetiporus* restriction groups (LRGs). SSIs from six collections of LRG II were completely compatible. SSIs from four LRG III collections from western North America were completely compatible, but those from a fifth collection of LRG III, from Michigan, were only partially compatible with those from the western LRG III collections. LRG II SSIs were incompatible with LRG III SSIs, and SSIs of these LRGs were incompatible with SSIs of *L. sulphureus, L. cincinnatus,* and LRG VI. SSIs from the lone collection of LRG VI were compatible with those of *L. sulphureus,* which also contains LRGs I and VII. Thus, *L. sulphureus* s. 1. is composed of four *Laetiporus* incompatibility groups (LIGs): *L. sulphureus* s. s., *L. cincinnatus,* LIG II, and LIG III. The LIGs differ in host, pore layer color, geographical distribution, and LRG. Pairings of tester SSIs from each of the LIGs with SSIs from an additional 42 collections of *Laetiporus* confirm the mutual exclusiveness of the LIGs and the association of the LIGs with the LRGs as well as other characteristics. Therefore, these four LIGs are worthy of recognition at the species level.

Key Words: allozyme, *L. cincinnatus, L. sulphureus,* pairings, Polyporaceae

INTRODUCTION

Laetiporus sulphureus (Fr.) Murr. s. 1. in North Americaica has recently been divided into seven groups based upon restriction fragment length polymorphisms in the ITS region of the nuclear ribosomal DNA (Banik et al 1998). Individuals within each *Laetiporus* restriction group (LRG) usually share the same pore color, host preference, and fruiting position. Three of these groups are easily distinguished using differences in these characters.

LRGs I, VI, and VII have yellow pores and fruit on the stumps and trunks of hardwood trees. Only one collection of LRG VI is known (from Wisconsin), but groups I and VII occur throughout the eastern United States from Mississippi to New York (Banik et al 1998). Of the LRGs described, only LRG VII was not unique, because its restriction pattern appeared to be a combination of those present in LRGs I and VI (Banik et al 1998). Pairings between single spore isolates (SSIs) of LRG I and LRG VII demonstrated them to be compatible. Compatibility was defined as fusion of the two SSIs accompanied by a change in culture morphology to a form with increased density and pigmentation (IDAP) (see FIG. 1A) when compared with the SSIs. The occurrence of the IDAP reaction was correlated with heterokaryosis as indicated by the presence of heterodimeric glucose-6-phosphate isomerase (GPI) activity in the resulting cultures (Banik and Burdsall 1999). Thus, LRGs I and VII are conspecific and were labeled *L. sulphureus* s. s., because they agree with the original description of this species (Bulliard 1809, p 347, pl 429). The compatibility of LRG VI could not be examined because SSIs were lacking.

Fruit bodies from LRG IV have white pores and typically arise from soil near the base of hardwood trees, especially *Quercus* spp., on which the fungus has been associated with a root rot (Rosen 1927). These characteristics fit the fungus described as *Polyporus cincinnatus* Morgan (Morgan 1885). Because of these characteristics and the lack of restriction patterns indicative of genetic exchange between LRG IV and any of the other LRGs, this taxon was transferred to *Laetiporus* as *L. cincinnatus* (Morgan) Burdsall, Banik & Volk (Banik et al 1998) The position of LRG IV as the species *L. cincinnatus* was further strengthened by subsequent incompatibility data in culture. By using GPI allozyme markers, it was determined that SSIs of *L. sulphureus* (LRGs I and VII) and *L. cincinnatus* did not undergo heterokaryotization

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TABLE I. Field data for 21 collections of *Laetiporus* from which single spore isolates were obtained for use in determining *Laetiporus* incompatibility groups

Species	*Laetiporus* restriction group	Collection number	Host	Pore color	Fruiting position	Location
sulphureus	I	GR-12	hardwood	yellow	log	Grant Co., WI
sulphureus	I	NJ-2	*Quercus* sp.	yellow	trunk	Morris Co., NJ
sulphureus	I	TJV 95-84	*Prunus serotina*	yellow	trunk	Dane Co., WI
sulphureus	VII	CT-1	*Acer* sp.	yellow	stump	Darien Co., CT
sulphureus	VII	TJV 95-53	*Acer* sp.	yellow	trunk	Baraga Co., MI
sulphureus	VII	TJV 95-83	*Quercus* sp.	yellow	log	Dane Co., WI
cincinnatus	IV	DA-37	*Q. velutina*	white	soil	Dane Co., WI
cincinnatus	IV	WS-1	—	white	soil	Washington Co., WI
cincinnatus	IV	TJV 95-49	*A. saccharum*	white	soil	Sauk Co., WI
Unnamed	II	CA-7	*Eucalyptus* sp.	yellow	stump	Santa Cruz Co., CA
Unnamed	II	CA-13	*Quercus* sp.	yellow	trunk	Napa Co., CA
Unnamed	II	OR-2	*Quercus* sp.	yellow	trunk	Marion Co., OR
Unnamed	II	OR-3	*Quercus* sp.	yellow	trunk	Marion Co., OR
Unnamed	II	TJV 95-106	hardwood	salmon	trunk	Jefferson Parish, LA
Unnamed	II	TJV 95-107	hardwood	salmon	trunk	Jefferson Parish, LA
Unnamed	III	AK-1	*Picea* sp.	yellow	stump	Anchorage, AK
Unnamed	III	CA-8	*Sequoia sempervirens*	yellow	trunk	Mendocino Co., CA
Unnamed	III	JAM-1	*Tsuga heterophylla*	yellow	trunk	Ketchikan, AK
Unnamed	III	MI-7	*T. canadensis*	yellow	log	Marquette Co., MI
Unnamed	III	NV-2	*Pinus contorta*	yellow	trunk	Washoe Co., NV
Unnamed	VI	DA-41	*Salix nigra*	yellow	trunk	Dane Co., WI

when paired with each other. This was morphologically expressed by the formation of a dense line of heavily pigmented mycelium between paired SSIs of the two species (see FIG. 1E). The IDAP reaction occurred in pairings between SSIs of all collections of *L. cincinnatus* tested, indicating they were completely compatible (see FIG. 1B). However, heterokaryosis could not be confirmed because all collections of this species were of the same allotype for the enzyme system used. For both *L. sulphureus* and *L. cincinnatus*, the pattern of the IDAP reaction in pairings between sibling SSIs suggests their mating system is unifactorial (Banik and Burdsall 1999).

LRG II fruits on the trunks and stumps of hardwoods, usually *Quercus* or *Eucalyptus*. It occurs in moderate climates, having been previously collected only in the extreme southern United States and coastal California. It is the only LRG to express variation in pore color, with three collections from Louisiana having white pores and all other collections have yellow pores (Banik et al 1998).

LRG III is the only LRG to occur on conifers, fruiting on stumps and living and dead trunks and having a yellow pore layer. It is common in old-growth conifer forests of the western United States. However, it is rare in the eastern United States, presumably because of the limited occurrence of these forests (Banik et al 1998). (An anonymous reviewer of this manuscript has indicated that *Laetiporus* is common on second-growth conifers in the northeastern United States, but we have no data on such occurrences.)

The purpose of this study was to investigate the relationship of LRGs II, III, and VI to each other and to *L. sulphureus* and *L. cincinnatus* using the established pairing protocol and allozyme analysis.

MATERIALS AND METHODS

SSIs were obtained, using the technique described by Banik and Burdsall (1999), from six collections of LRG II, five of LRG III, and one of LRG VI (TABLE I). Restriction group determinations were made using criteria previously identified (Banik et al 1998). Twelve SSIs from each collection were paired in all combinations with their sibling SSIs, and the results were interpreted using the protocol in Banik and Burdsall (1999).

Based on the results of the initial pairings, two SSIs of different mating types were chosen as testers from each collection of LRG II and LRG III and paired with the other tester SSIs from within the same LRG. These parings were replicated twice and rated visually as described previously.

Each SSI chosen was subjected to allozyme analysis for the dimeric enzyme glucose-6-phosphate isomerase (GPI)

as in Banik and Burdsall (1999). Differences in allotype between SSIs from selected collections allowed for verification of the pairing results. After the pairings had incubated for 5-7 d, three hyphal tips were cut with the aid of a dissecting microscope from the central interaction zone of each pairing between non-identical allotype SSIs. The hyphal tips were placed on 2.5% w/v gallic acid potato dextrose agar medium (Difco, Detroit, Michigan), and the resulting mycelia were analyzed for allotype GPI activity (Banik and Burdsall 1999).

To determine the incompatibility between LRGs, six SSIs of LRG II, six of LRG III, four of LRG III isolate MI-7, and four of LRG VI were paired with the selected SSIs of the other LRGs as well as with six SSIs each of *L. cincinnatus* and *L. sulphureus* (TABLE I). These pairings were conducted as described previously and replicated twice. Allozyme analysis was performed on hyphal tip cultures from a subset of all pairings in which a dense line did not form between the paired SSIs. Allozyme analysis of a subset of the pairings exhibiting a dense line was accomplished by analyzing two mass mycelium samples, one from each side of the line, for GPI allotype activity (Banik and Burdsall 1999).

To more comprehensively examine the *Laetiporus* incompatibility groups (LIGs) identified in previous experiments, three SSIs of each LIG were paired against two SSIs randomly chosen from 42 different collections. These 42 collections had not been involved in any other pairings, but the LRGs of 37 of them were previously determined by the method described by Banik et al (1998). The collection data for these 42 collections are available from the authors. The pairings were performed using the method described previously (Banik and Burdsall 1999).

RESULTS

Intra LRG II pairings.—Pairings between sibling SSIs from collections of LRG II expressed IDAP reactions (FIG. 1C) in a pattern consistent with a unifactorial mating system. In five of the collections, the fit to a bipolar system was exact and in one collection two pairings were rated as fusions that were expected to be IDAP. The numbers of SSIs in each collection belonging to the two mating types were as follows: 5 and 7 for CA-13, 4 and 8 for TJV 95-107, 7 and 7 for CA-7, 5 and 7 for OR-3, and 3 and 9 for OR-2. Pairings that did not exhibit an IDAP reaction usually fused (FIG. lG), but in a few pairings the SSIs remained separate without the formation of a pigmented line between them (separate without a line, SWAL) (FIG. 1H). Two GPI allotypes were identified, with SSIs of collections TJV 95-106, TJV 95-107 and OR-3 being type C, and CA-7, CA-13 and OR-2 type I (FIG. 2).

Fifty-four of 66 nonself pairings between two single spores from each LRG II collection exhibited the IDAP reaction for both replicates, and 12 were rated as IDAP for one replicate and either as a fusion or as SWAL for the other (FIG. 1C, G, H). Twenty-five pairings exhibiting the IDAP reaction for both replicates, and 7 pairings showing this reaction for only one replicate were tested for GPI activity and all were heterodimeric (TABLE II).

Intra LRG III pairings.—The patterns of the IDAP reactions in sibling SSI pairings of LRG III collections JAM-1 and AK-1 suggest bipolarity, although the IDAP reaction was not as pronounced as that seen in pairings of the other LRGs (FIG. 1D). In JAM-1 there were three SSIs of one mating type and nine of the other. Two pairings that were expected to be rated as IDAP appeared only to fuse. In AK-1, five SSIs were of one mating type and six of the other. One AK-1 SSI did not produce the IDAP reaction in any pairing. Excluding this isolate, there was one pairing rated as a fusion that was expected to express the IDAP reaction. The pattern of the reaction types of the other three LRG III collections was not easily determined. All but two SSIs from collection CA-8 fused with each other. One of these formed a dense line with all the other isolates, and the other expressed the IDAP reaction with all the other isolates except for the dense line forming isolate. Three SSIs of NV-2 produced the IDAP reaction with six others, except that one pairing was rated as a fusion that was expected to be an IDAP. Three SSIs of this collection fused with all the other isolates without the occurrence of an IDAP. All 12 SSIs of MI-7 fused with each other. Three GPI allotypes were present in LRG III SSIs. Isolates of AK-1, JAM-1, and NV-2 were type B, CA-8 was type A, and MI-7 was type C (FIG. 2).

None of the 16 pairings between two SSIs of MI-7 and the SSIs of the other four collections of LRG III exhibited the IDAP reaction for either replicate. These pairings mostly exhibited fusion reactions, but some were SWAL or unreadable after 7 d of incubation. After 2 wk, almost all these pairings exhibited a dark line between the culture arising from the outer MI-7 plug and the rest of the culture. These pairings were difficult to interpret because the MI-7 SSIs grew much slower than the SSIs from the other collections. Allozyme analysis of cultures arising from excised hyphal tips of these pairings indicated heterodimeric GPI activity in three pairings involving one of the MI-7 SSIs and one SSI from each of three other LRG collections. The remaining 13 pairings involving MI-7 did not possess heterodimeric GPI activity in 28 different hyphal tip cultures, with at least one culture analyzed from each pairing. Because of the inconsistent results of the pairings of MI-7 with the other members of this LRG, it was treated separately in the inter-LRG pairing test (TABLE II).

Twenty-three of 24 pairings between the SSIs from

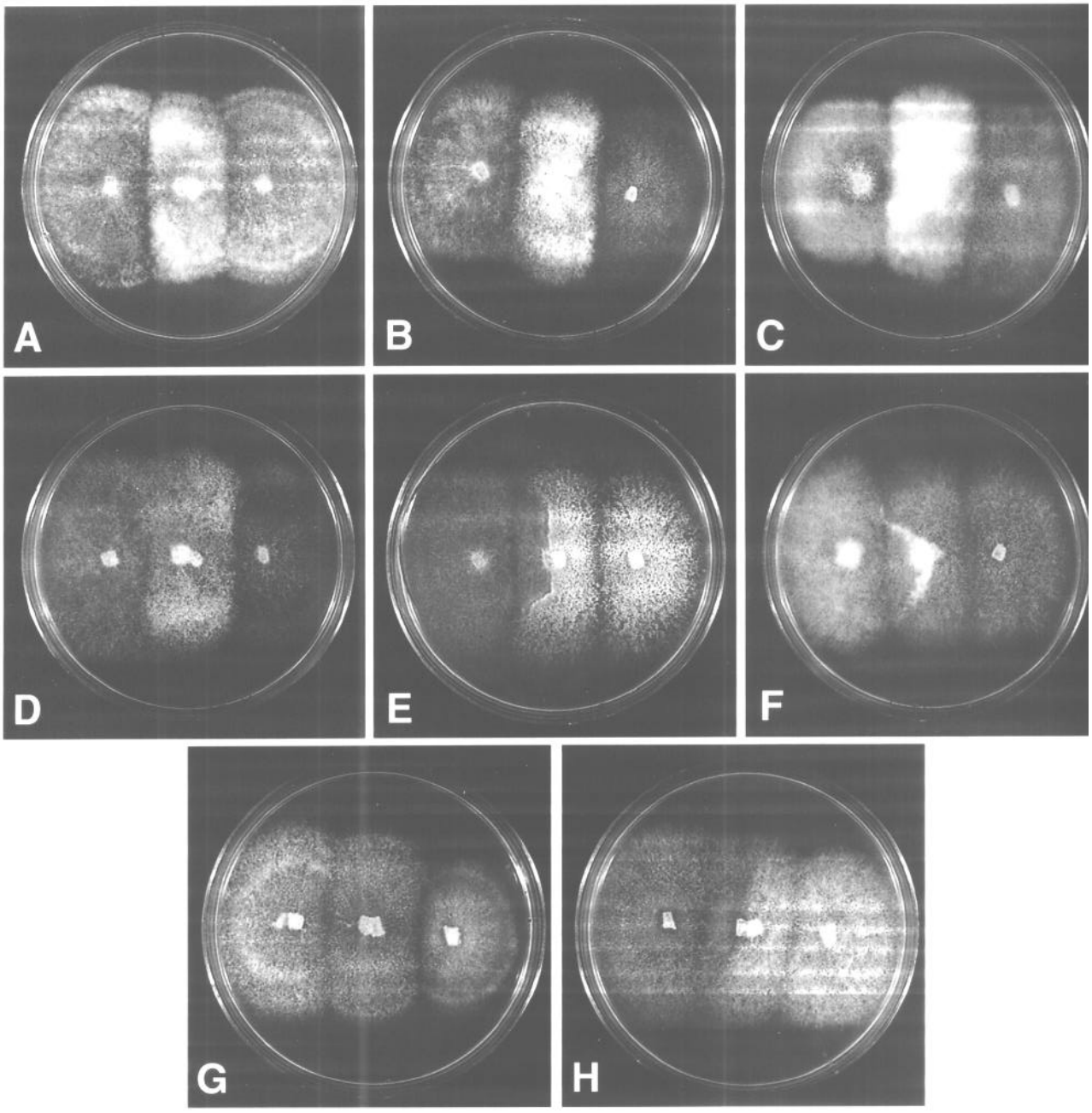

FIG. 1. Culture morphologies observed in pairings between single spore isolates (SSIs) of *Laetiporus* incompatibility groups (LIGs). Increased density and pigmentation (IDAP) (A-D) reactions observed only within LIG pairings between two SSIs of A. *L. sulphureus,* B. L. *cincinnatus,* C. LIG II, or D. LIG III. The formation of a dense line (E, F) separating the two SSIs was observed consistently in between species pairings, and G. fusions and H. separations without a line (SWAL) were only observed within species pairings. In each Petri dish, nonself pairings involve the two central plugs, while self pairings occur between the outer plugs and the nearest central plug.

different LRG III collections, excluding MI-7, were rated as IDAP for both replicates (FIG. 1D). The remaining pairing was rated IDAP for one replicate and SWAL for the other (FIG. 1H). Allozyme analysis of 12 pairings indicated heterodimeric GPI activity. No other pairings in this LRG could be analyzed because the collections were the same allotype (TABLE II).

Intra LRG VI pairings.—Twenty-four of the 66 nonself sibling pairings of DA-41 (LRG VI) SSIs exhibited an IDAP reaction (FIG. 1A). Of the remaining 42 nonself pairings, 12 were SWAL (FIG. lH), 2 remained separate with dense line formation between the SSIs (FIG. 1E), and in 28 the two SSIs fused (FIG. 1G). No distinct pattern of mating-type reactions was

TABLE II. Number of pairings exhibiting each of four reaction morphologies following 1-wk incubation between single spore isolates (SSIs) of *Laetiporus* restriction groups (LRG) and the frequency of recovery of GPI allozyme patterns indicative of heterodimeric activity

LRGs paired	Total pairings[a]	Reaction type[b]				Heterodimeric GPI/pairs tested
		IDAP	Fusion	SWAL	Dense line	
II × II	78	55-65	21-13	2-0	0-0	32/32
III[c] × III	55	28-26	12-25	1 4	0-0	15/28
I, VII[d] × II	36	0-0	0-0	0-0	36-36	0/16
I, VII × III	36	0-0	0-0	0-0	36-36	0/12
I, VII × VI	24	19-23	4-1	1-0	0-0	18/18
I, VII × MI-7	24	0-0	0-0	0-0	24-24	0/9
IV[e] × II	24	0-0	0-0	0-0	36-36	0/12
IV × III	36	0-0	0-0	0-0	36-36	0/6
IV × VI	24	0-0	0-0	0-0	24-24	—
IV × MI-7	24	0-0	0-0	0-0	24-24	0/8
II × III	36	0-0	0-0	0-0	36-36	0/13
II × VI	24	0-0	0-0	0-0	24-24	0/6
II × MI-7	24	0-0	0-0	0-0	24-24	0/8
III × VI	24	0-0	0-0	0-0	24-24	0/4
III × MI-7	12	0-0	0-0	0-0	12-12	3/8
VI × MI-7	16	0-0	0-0	0-0	16-16	0/5

[a] II × II and III × III include 12 and 10 self pairings, respectively.

[b] Results are from two different replications. Reaction morphologies observed are as follows: IDAP—an increase in density and pigmentation over that of the SSIs, fusion—SSIs fuse without a change in morphology; SWAL—SSIs remain separate without a dense line being formed between them, dense line—SSIs remain separate with the formation of a dense pigmented line between them.

[c] Within LRG III SSIs of collection MI-7 reacted inconsistently with those of the other collections and 14 pairings in the first replicate were unreadable after l-week incubation. All pairings within LRG III that did not express heterodimeric GPI activity involved SSIs from collection MI-7.

[d] LRGs I and VII were previously shown to be compatible and are considered to belong in *L. sulphureus.*

[e] LRG IV is *L. cincinnatus.*

discernable among the pairings. The SSIs of DA-41 were all GPI allotype B (FIG. 2).

Pairings between LRGs.—All pairings between single spore tester isolates of *L. sulphureus* (LRGs I and VII) and LRG II, LRG III, or MI-7 developed a dense line between the SSIs and none of the pairings analyzed for GPI activity possessed heterodimeric patterns (TABLE II). Of the 24 pairings between LRG Vl and *L. sulphureus,* 18 displayed an IDAP reaction for both replicates. Thirteen of these pairings were tested for GPI activity, and all were heterodimeric (FIG. 2). Five of the pairings between LRG VI and *L. sulphureus* either fused or exhibited a SWAL reaction for one replicate and expressed an IDAP reaction in the other. Four of these pairings were tested for GPI activity, and all had a heterodimeric allozyme pattern. The last pairing exhibited a fusion reaction for both replicates and possessed heterodimeric GPI activity (TABLE II).

All pairings between *L. cincinnatus* and LRGs II, III, VI, or isolate MI-7 resulted in the dense line reaction between the SSIs in both replicates (FIGS. 1E, F). No heterodimeric GPI activity was detected in pairings between *L. cincinnatus* and LRG II, LRG III, and isolate MI-7. No pairings could be analyzed between *L. cincinnatus* and LRG VI because they have the same allotype (TABLE II). All pairings between LRG II SSIs and SSIs from LRGs III, and VI, and isolate MI-7 exhibited a dense line reaction, and no heterodimeric GPI activity was detected (TABLE II). All pairings between SSIs of LRG VI and LRG III or collection MI-7 were characterized by the dense line reaction, and no heterodimeric GPI activity was detected (TABLE II).

The consistent occurrence of the dense line reaction in pairings between SSIs of LRG II, LRG III, *L. sulphureus,* and *L. cincinnatus* confirm that each group is incompatible with the others. Therefore, LRGs II and III are designated as *Laetiporus* incompatibility groups (LIG) II and III, respectively. LRG VI is completely compatible with *L. sulphureus* and is so labeled.

In addition to the 16 pairings between SSIs of MI-7 and the other collections of LRG III performed in

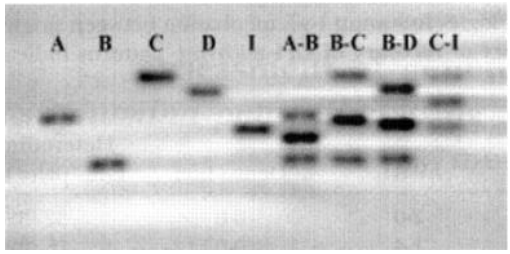

FIG. 2. Representative GPI allozyme patterns obtained from isolates of *Laetiporus* by electrophoresis on cellulose acetate gels. Homodimeric patterns of *L. sulphureus* (lanes A, B, C, D) L. *cincinnatus* (lane B) LRG II (lanes C, I) and LRG III (lanes A, B, C) are shown as well as heterodimeric patterns obtained from pairings between single spore isolates within each species (lanes A-B, B-C, B-D, C-I).

the intra-LRG tests, another 12 pairings were conducted using two different SSIs of MI-7 during the inter-LRG tests. None of these pairings exhibited an IDAP reaction, but all formed a dense line after 2 wk incubation. Isozyme analysis of masses of mycelium taken from each side of the dense line for eight pairings indicated no heterodimeric GPI activity in five of these pairings. In three pairings, heterodimeric activity was detected on the LRG III side of the pairing but not on the MI-7 side of the pairing (TABLE II). These data do not conclusively show MI-7 and LIG III to be compatible, and thus MI-7 will be provisionally excluded from LIG III.

Pairings between LIG tester isolates and untested SSIs.—SSIs from 41 of 42 unidentified *Laetiporus* collections produced the IDAP reaction (i.e., were compatible) with only one LIG when paired with tester SSIs from the four LIGs. Twenty-five of these collections were compatible with *L. sulphureus* tester isolates, 13 with *L. cincinnatus* tester isolates, and 3 with tester isolates of LIG II. Two hundred forty-six pairings were read between SSIs from unknown collections and the tester isolates from the LIG with which they were compatible. Of these pairings, 216 (88%) gave the IDAP reaction, with the remaining reactions being either fusions or SWAL. In no instance did fewer than four of the six pairings between the two SSIs from the unknown collection and the three tester isolates from the incompatibility group it was identified with produce the IDAP reaction. All 1008 pairings between the unknown SSIs and the tester isolates from LIGs with which they had no IDAP reaction produced a dense line between the two isolates.

Of the 25 collections identified as *L. sulphureus*, 22 had previously been categorized as belonging to either LRG I or VII, and the LRGs of the other 3 were not determined. The LRG of 12 of the 13 collections identified as *L. cincinnatus* was determined and found to be LRG IV. The three collections identified as LIG II all belong to LRG II.

SSIs from one collection (CA-19) did not produce the IDAP reaction with any of the tester isolates. It did produce the dense line reaction with all tester isolates, except for one isolate of LIG III with which it produced the SWAL reaction. Allozyme analysis of this pairing could not be conducted because the test isolates were the same allotype. Allozyme analysis of the four pairings between LIG III and CA-19 that could be analyzed gave no indication of heterodimeric GPI activiy. The LRG of this collection was not previously known, but has since been determined and found to be different from the other LRGs. This fruiting body has yellow pores and was on the trunk of a *Quercus* sp. in the Sierra Nevada Mountains of Mariposa Co., California.

DISCUSSION

Allozyme analysis of pairings between SSIs from different collections of LRG II confirm the association of the IDAP morphology with heterokaryosis as indicated by the occurrence of banding patterns associated with heterodimeric GPI activity. Complete compatibility between the LRG II collections studied indicates the presence of a multiallelic mating system as was previously documented for *L. sulphureus* and *L. cincinnatus.* The distribution of IDAP reactions found within collection pairings suggests a unifactorial mating system that was also observed for the other two species (Banik and Burdsall 1990).

The results of the pairings between SSIs of different LRG III collections and subsequent allozyme analysis show that four of the collections are completely compatible. Two of these collections have a bipolar mating pattern when the IDAP reaction is used as an indicator of positive pairings. The mating system of the other two collections is less certain, but IDAP reactions were observed in pairings within each collection. However, no IDAP reactions were observed in sibling pairings of LRG III collection MI-7. SSIs of this collection also did not produce the IDAP reaction when paired with SSIs of the other LRG III collections. However, allozyme analysis indicated that nuclear combination occurred in 7 of 24 pairings tested between MI-7 and the other LRG III collections. The significance of this amount of compatibility is unknown, especially when compared with the complete compatibility between collections in the other LIGs in the genus. MI-7 is the only member of LRG III collected from east of the Rocky Mountains, and the eastern distribution of LRG III may be limited by a lack of old-growth conifer stands. The SSIs of MI-7 are also significantly different in growth habit

from those of the SSIs of the other LRG III collections. Growth of MI-7 isolates is 25% of the rate of the other isolates, and they are uniformly bright orange versus nearly white for SSIs from the other collections. These culture characteristics make pairing reactions with MI-7 difficult to interpret. The characteristics of the SSIs could be an indication that they are not homokaryotic but heterokaryotic, perhaps as a result of homothallism or amphithallism. This would explain the complete fusion observed between the SSIs of this collection, since they would see each other as self. In addition, the allozyme analysis indicates that nuclear migration does not occur into SSIs of MI-7 from SSIs of the other LRG III collections. This might be expected if the MI-7 mycelium is heterokaryotic. It seems prudent to segregate MI-7 from the rest of the LRG III collections until more collections of this potentially distinct taxon are studied.

Results of pairings of single spore tester isolates from each of the four LIGs with SSIs from an additional 42 collections are consistent with the concept that the LIGs are mutually exclusive. In addition, the strong correlation between the LRGs and their respective LIGs is further demonstrated.

Laetiporus sulphureus is composed of collections belonging to LRGs I, VI, and VII. The inclusion of LRG VI was predicted because the restriction pattern of LRG VII appeared as a combination of those seen in LRGs I and VI (Banik et al 1998). *Laetiporus cincinnatus* is composed only of collections of LRG IV, and LIG II collections all belong to LRG II. LIG III consists of collections of LRG III from western North America, including California, Idaho, Oregon, Washington, British Columbia, and Alaska. No validly published species names appear to be associated with either LIG II or III.

Of the seven LRGs originally delimited, all but LRG V, which was not studied here, have been associated with a LIG. Spores of LRG V have failed to germinate, making pairing tests impossible, Attempts at di-mon pairings as a means to determine its relationship with the other LIGs have been unproductive. One collection (CA-19) was identified in this study as belonging to a previously unidentified LRG. Unfortunately, only three SSIs of this collection exist. Additional work is warranted with these two LRGs and eastern isolates of LRG III to determine their taxonomic status.

Based on incompatibility data, restriction enzyme analysis of the ribosomal DNA, macromorphological data, and ecological data, the species *L. sulphureus* has now been shown to be a complex of at least four taxa. Two of these taxa, *L. sulphureus* and *L. cincinnatus,* were previously described. The immediate goal of our future research will be to describe collections of LIGs II and III as new species.

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