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SEROLOGICAL DIFFERENTIATION OF TWO FORMS OF *PHELLINUS WEIRII*

MARK T. BANIK JENNIFER A. PAUL, HAROLD H. BURDSALL, JR., AND MARK E. COOK

USDA Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, Wisconsin 53705-2398

ABSTRACT

Two forms of *Phellinus weirii* exist in North America that are morphologically similar, and identification of these isolates is often difficult. The purposes of this study were 1) to assess the potential of using an enzyme-linked immunosorbent assay (ELISA) as a rapid means of separating these two forms in culture and 2) to determine the relatedness of the two forms and *P. sulphurascens* from Asia using ELISA and western blots from polyactylamide gel eletrophoresis. Separation of the two North American forms was achieved using chicken antibodies specific to one of the forms in ELISA. In serological tests, *P. sulphurasens* and the Douglas-fir form react similarly to one another but differently from the redcedar form. These data support previous suggestions that the two North American forms of *P. weirii are* distinct species. The assertion that *P. sulphurascens is* nonspecific with *P. weirii is* supported only when *P. sulphurascens is* compared serologically with Douglas-fir form isolates.

Key Words ELISA, *Phellinus,* serology, western blots

Phellinus weirii (Murr.) Gilbn. causes a butt rot of *Thuja plicata* Donn. *ex* D. Don (western redcedar) and a root rot *of Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir). The fungus is also known to occur on several other conifer tree species in northwestem North America, including *Tsuga heterophylla* (Raf.) Sarg. (western hemlock), *Picea sitchensis* (Bong.) Carr. (Sitka spruce), and several *Abies* and *Pinus* species (Clark, **1958).**

The fungus was originally described from western redcedar by Murrill **(1914).** Based on similarities in fruiting bodies, host decay type, and cultural characteristics, Mounce et al. **(1940)** concluded that *P. weirii,* "or a form of it," was also responsible for decay of Douglas-fir. Several researchers have advocated the occurrence of two forms of *P. weirii* (Buckland et al., **1954;** Clark, **1958;** Larsen and Lombard, **1989)** distinguished chiefly by differences in fruiting habit, host specificity, and cultural characteristics. The Douglas-fir form is annual and occurs on many conifers but rarely on western redcedar. The redcedar form is perennial and occurs almost exclusively on western redcedar. Cultures from redcedar are considerably darker in color than those of the Douglas-fir form and produce numerous columnar tufts (Larsen and Lombard, **1989).**

Angwin **(1989)** divided North American *P. weirii* isolates into two biological species based on cultural confrontations and electrophoretic banding patterns. These biological species corresponded to the Douglas-fir and redcedar forms described by other researchers. He further suggested the occurrence of an additional Asian biological species baaed on cultural confrontation tests involving two isolates from Japan and isolates of the redcedar and Douglas-fir forms. Electrophoretic data indicated that the two North American forms were more closely related to each other than they were to the Japanese isolates (Angwin, **1989).**

Kotlaba and Pouzar **(1970)** concluded that the Asian species *P. sulphurascens* ***is*** nonspecific with *P. weirii* baaed on similarities in fruiting body morphology. They also inferred from fruiting body microstructure that the species was a member of the genus *Inonotus.* However, this placement is not widely accepted.

Serological techniques have been employed by several researchers to help resolve differences between species. Aldwell et al. **(1985)** demonstrated antigenic differences between species of the Endogonaceae using ELISA. Ferguson et al. **(1988)** used ELISA to separate 14 phytopathogenic fungi into their appropriate taxonomic groups. In addition, Lung-Escarmant and Duttez **(1979)** used ELISA to distinguish four species of *Armillaria.*

The objective of this paper is to report a rapid means of separating the North American forms of *P. weirii* using chicken antibodies in an en-

zyme-linked immunosorbent assay (ELISA). In addition. serological data on the relatedness of North American *P. weirii* forms and Asian isolates are presented.

MATERIALS AND METHODS

Antibody production and purification. – Fungal material injected into chickens was grown in liquid culture according to the protocol previously described (Burdsall et al.. **1990).** Immunogen was prepared by grinding 30 mg lyophilized mycelium of *P. weirii* isolate FP-133613 (Douglas-fir) with 11 ml phosphate buffered saline (PBS) in a 15-ml ground glass tissue homogenizer. The resulting homogenate was centrifuged at 14 K × *g* for 15 min. The supernatant was then recentrifuged at 30 K × *g* for 2 h. The resulting supernatant was brought to 85% saturation with ammonium sulfate. The precipitate was collected by centrifugation at 14 K × *g* for 10 min. redissolved in 1.5 ml PBS, and dialyzed overnight against 500 ml PBS. The resulting antigen contained 228 µg/ml of protein as determined using a Bradford **(1976)** protein assay. The antigen was used as immunogen to inject a White Leghorn laying hen. The antibodies were purified from the yolks of eggs laid the day of injection and 21 days after injection (DAI). The 21 -DAI antibody preparation was termed DfAb.

ELISA antigen preparation and test procedure.– Fungal cultures used as a source of antigenic material for ELISA tests were grown on a chemically defined medium (CD medium) developed by Leatham **(1983).** The liquid medium was prepared double strength, and an equal volume of sterilized 4% agar was added after autoclaving. Petri dishes containing this medium were seeded in the center with agar plugs from stock cultures of each of the isolates in **TABLE** I. A 5-mm-diam agar plug was cut from the margin of the colonies after 1-4 wk of incubation at room temperature and ground with 4 ml of 0.05 M carbonate buffer (pH 9.6) in a 15-ml ground glass tissue homogenizer. This material was then frozen (-20 C) and used as the antigen for ELISA tests.

The ELISA procedure used was that deseribed by Burdsall et al **(1990).** The appropriate antigen (200 µl), prepared as stated, was added to each ELISA plate well and incubated overnight at 6 C. After removal of the antigen, 125 µl of Blotto (Johnson et al., **1984)** was added to each well as a blocking agent. After incubating for 1 h at room temperature, the Blotto was removed and chicken antibody was added.

Antibodies were cross-absorbed prior to use with either CD agar or CD agar colonized by redcedar form isolate FP-134603. To cross-absorb with uncolonized agar, two 5-mm-diam CD agar plugs were added to 2 ml of PBS and Tween-20 (PBS-T) and ground in a 15-ml ground glass tissue homogenizer. To cross-absorb with FP-134603, one 5-mm uncolonized agar plug and one 5-mm colonized agar plug were ground in 2 ml PBS-T. DfAb cross-absorbed with FP-134603 was termed DfAbxaR. The chicken antibody of interest was then diluted 1: 100 in the appropriate PBS-T/agar plug solution and allowed to react at room temperature for 1 h prior to being placed in the plate. At this time. 100 µl of the appropriate antibody solution was placed in each well. in all tests, antibodies obtained from eggs laid the day of injection (day 0) were run as controls for eggs colleeted 21 DAI

After the chicken primary antibody was removed from the well, 100 µl of rabbit antichicken enzyme conjugated secondary antibody (ICN, Immuno Biological, Lisle, Illinois) was diluted 1:5000 in PBS-T and added to each well. After a 2-h incubation, the secondary antibody was removed and 100 µl of 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate solution (Kirkegaard and Perry Laboratones. Inc., Gaithersburg, Maryland) was placed in each well. The reactions were allowed to proceed to completion, and the optical densities (ODs) were determined at 630 nm with a Dynatech microtiter plate reader.

Comparison of Douglas-fir and redcedar forms of P. weirii *using ELlSA* **.—** To determine whether the two North American *P. weirii* forms can be serologicaIly distinguished using ELISA, DfAb was reacted with antigens from 55 *P. weirii* isolates **(**TABLE I). The antibody was reacted once with each isolate in a single microtiter plate well following the ELISA procedure outlined. The reaction of DfAbxaR with the 55 isolates was carried out twice.

ELISA reactions were visually rated positive or negative and the ODs were determined. The ODs from day 0 control eggs were subtracted from the ODs of the day 21 eggs for a given antigen. Means of the ODs were compared using Student's *t* -test. For statistical analyses of data with more than one replicate, the OD values were standardized by converting them to a percentage of the homologous reaction in each replicate. The averaged standardized means were then compared using Student's *t* -test.

Comparison of Douglas-fir, redcedar, and Asian form haploid isolates of P. weirii *using ELISA.* -To determine the serological relationships among three forms of *P. weirii*, three haploid isolates from each of two collections of the Douglas-fir (FP-134848, FP-134842), redcedar (FP-135675, FP-135684), and Asian form (3228, 3305) from Siberia (TABLE I) of *P. weirii* were grown as described. The isolates were reacted with DfAb or DfAbxaR using the ELISA procedure. The test was performed once for DfAb, three times with DfAbxaR, and the data were standardized and analyzed as before.

SDS-PAGE and Western blot procedure. – Fungal material used in electrophoresis was grown on cellophane-covered CD agar. Mycelial mats 6-10 days old were harvested and ground in a tissue homogenizer with sample buffer [1 ml 0.5 M Tris-HCl, pH 6.8; 0.8 ml glycerol: 1.6 ml 10%(w/v) sodium dodecyl sulfate (SDS): 0.4 ml 2- ß -mercaptoethanol; 0.2 ml 1% bromophenol blue]. Twenty milligrams (fresh weight) of mycelium was ground with 0.5 ml sample buffer. The resulting solution was placed in a test tube in a boiling water bath for 4.5 min and then centrifuged at 16 K × *g* for 10 min. Thirty microliters of the supernatant was placed in each well of a 12% SDS-polyacrylamide gel. Each gel was run with the inclusion of a low molecular weight standard (Bio-Rad Laboratories, Richmond, California) in at least one of the 10 lanes. Gels were run in a

TABLE I

COLLECTION DATA FOR THE *PHELLINUS* ISOLATES USED IN THIS STUDY

Isolate	Host	Location	Form[a]
13	*Pseudotsuga menziesii*	Oregon	Douglas-fir
36	*Pseudotsuga menziesii*	Washington	Douglas-fir
JH-50-WR-df	*Pseudotsuga menziesii*	Washington	Douglas-fir
JH-50-WR-1p	*Pinus contorta*	Washington	Douglas-fir
JH-53-Ba	*Picea sitchensis*	Oregon	Douglas-fir
104	*Pseudotsuga menziesii*	Idaho	Douglas-fir
121	*Pinus contorta*	Washington	Douglas-fir
195	*Pinus monticola*	British Columbia	Douglas-fir
197	*Picea sitchensis*	Oregon	Douglas-fir
199	*Tsuga heterophylla*	Oregon	Douglas-fir
219	*Abies concolor*	Washington	Douglas-fir
228	*Larix occidentalis*	Washington	Douglas-fir
G-7312	*Pseudotsuga menziesii*	Washington	Douglas-fir
DAOMF-9422	*Pseudotsuga menziesii*	British Columbia	Douglas-fir
FP-91601-T	*Pseudotsuga menziesii*	Washington	Douglas-fir
FP-105770-R	*Abies*	Idaho	Douglas-fir
FP-105771-R	*Abies*	Idaho	Douglas-fir
FP-133613-(A)Sp	*Pseudotsuga menziesii*	Oregon	Douglas-fir
Kropp 93BB-R	*Pseudotsuga menziesii*	Oregon	Douglas-fir
FP-134842-T	*Pseudotsuga menziesii*	Idaho	Douglas-fir
FP-134846-Sp	*Pseudotsuga menziesii*	Idaho	Douglas-fir
FP-134847-T	*Pseudotsuga menziesii*	Idaho	Douglas-fir
FP-134848-Sp	*Larix occidentalis*	Idaho	Douglas-fir
PW-DF-A3-10′	*Pseudotsuga menziesii*	Oregon	Douglas-fir
PW-DF-A3-20′	*Pseudotsuga menziesii*	Oregon	Douglas-fir
E6-DF-PW	*Pseudotsuga menziesii*	Oregon	Douglas-fir
E8-Th-P1	*Thuja plicata*	Oregon	Douglas-fir
PW-WRC-Rhodo-KF	*Thuja plicata*	Oregon	Douglas-fir
159	*Pseudotsuga menziesii*	British Columbia	Douglas-fir
FP-135682	*Thuja plicata*	Idaho	Redcedar
FP-135514-Sp	*Tsuga heterophylla*	Idaho	Redcedar
FP-134657-T	*Larix occidentalis*	Idaho	Redcedar
169	*Thuja plicata*	British Columbia	Redcedar
175	*Thuja plicata*	British Columbia	Redcedar
204	*Thuja plicata*	Idaho	Redcedar
206	*Abies grandis*	Idaho	Redcedar
214	*Thuja plicata*	Idaho	Redcedar
225	*Thuja plicata*	Washington	Redcedar
232	*Thuja plicata*	Alaska	Redcedar
OKM-4742-Sp	*Thuja plicata*	Idaho	Redcedar
FP-134614-Sp	*Thuja plicata*	Idaho	Redcedar
FP-134652-Sp	*Thuja plicata*	Idaho	Redcedar
FP-134603-Sp	*Thuja plicata*	Idaho	Redcedar
FP-134604-Sp	*Thuja plicata*	Idaho	Redcedar
FP-134951-Sp	*Thuja plicata*	Idaho	Redcedar
PW-Cedar-Sicar	*Thuja plicata*	British Columbia	Redcedar
FP-134801-Sp	*Thuja plicata*	Idaho	Redcedar
AKPw Het-Paul no. 2	*Thuja plicata*	Alaska	Redcedar
AKW Het-Paul1B	*Thuja plicata*	Alaska	Redcedar
FP-135440-Sp	*Thuja plicata*	Idaho	Redcedar
FP-135422-Sp	*Thuja plicata*	Idaho	Redcedar
FP-135667-T	*Thuja plicata*	Idaho	Redcedar
FP-135668-T	*Thuja plicata*	Idaho	Redcedar
171	*Abies lasiocarpa*	British Columbia	Redcedar
170	*Abies grandis*	British Columbia	Redcedar
FP-135675	*Thuja plicata*	Idaho	Redcedar
FP-135684	*Thuja plicata*	Idaho	Redcedar
3305[b]	Conifer log	Maise, Siberia	Asian
3228	Hardwood log	Maise, Siberia	Asian

[a] Isolate forms are those assigned by Clark (1958) or Larsen (pers. comm.). Isolates from other collections were assigned to a given form based on the host from which they were taken.

[b] Siberian isolates provided courtesy of Dr. Ronald H. Petersen, University of Tennessee, Knoxville.

Mini-Protean II[1] (Bio-Rad Laboratories) dual slab cell using a running buffer consisting of 3 g Tris base, 14.4 g glycine. and 1 g SDS per liter. The gels were run at 200 constant volts until the buffer front reached the bottom of the gel, approximately 40 min.

Following electrophoresis. the gels were equilibrated in transfer buffer (25 mM Tris base, 192 mM glycine, pH 8.3) for 15-30 min. The proteins were then electrophoretically transferred onto nitrocellulose paper (NCP) using a Mini Trans-Blot cell (Bio-Rad Laboratories) containing transfer buffer at 100 constant volts for 1 h. Blotted NCP was blocked in Blotto overnight at 6 C. Following blocking, the NCP was rinsed with distilled water and probed with the antibody of interest diluted 1:100 in Tris buffered saline plus Tween-20 (TTBS). If the antibody was cross-absorbed, the procedure described for ELISA was followed. After incubating for 2 h at room temperature, the NCP was rinsed with TTBS and treated with enzyme conjugated secondary antibody diluted 1:5000 in TBS (TTBS without Tween-20) containing 1% gelatin. After a 2-h incubation at room temperature, the blot was rinsed with TTBS and developed with 4ClN (Sigma Chemical) substrate [10 ml methanol containing 30 mg 4-chloro-l-naphthal, 50 ml triethanolamine buffer (2.8 ml triethanolamine, 17 ml 1 M HCl, pH 7.5), and 25 µl 30% hydrogen peroxide] at room temperature for 10-60 min.

Comparison of Douglas-fir and redcedar forms of P. weirii *using Western blots.* – To determine the serological relationships among proteins of the North American *P. weirii* forms, six isolates of the Douglas-fir form (FP-133613, E-6, G7312, 11189,6029, 6024) and five of the redcedar form (FP-134603, FP 134614, 11223, 11840, 134801) (TABLE I) were prepared for electrophoresis. Each isolate was placed in a single gel lane and the blot was probed with DfAb. Lanes within blots were visually compared for similarities in banding patterns.

Comparison of haploid isolases from Asia, redcedar form, and Douglas-fir form of P. weirii *using Wesrern blots.* - To determine the serological relationship among proteins of three forms of *P. weirii*, the following haploid isolates were prepared for electrophoresis 134848 ss-1146, 134842 SS-1137 (Douglas-fir): 135684 ss-21, 135675 ss-20 (redcedar); and 3228 ss-3, 3228 ss-7, 3305 ss-6, and 330555-9 (Asia). Each isolate was placed in a single gel lane, and the resulting blot was probed with DfAb. Lanes within blots were compared for similarities in banding patterns.

RESULTS

Comparison of Douglas-fir and redcedar forms of P. weirii *using ELISA.* -The reactions of 26 *P. weirii* isolates with DfAbxaR were visually rated negative, and 29 were rated positive. Those scored as negative correspond to the isolates designated as the redcedar form based on other criteria. The 29 isolates scored as positive are believed to be the Douglas-fir form based on other criteria. Other criteria as used in this context include collection data, cultural characteristics, and decay characteristics. Reactions between all of the isolates and DfAb were not distinct enough to rate visually.

The ODs of the reactions of the 29 Douglas-fir isolates visually scored as positive using DfAbxaR ranged from 0.209 to 0.526 when reacted with DfAb with a mean of 0.381 ± 0.038 [95% confidence interval (CI)]. The ODs of the reactions between DfAb and the 26 redcedar isolates scored as negative with DfAbxaR ranged from 0.103 to 0.504 with a mean of 0.279 ± 0.038. The two means were significantly different by Student's *t* -test ($p = 0.0003$).

The ODs of the reactions of the 26 redcedar isolates with DfAbxaR over two runs ranged from 0.008 to 0.088 (TABLE II). The values for the Douglas-fir isolates ranged from 0.113 to 0.431 (TABLE II). When the data were standardized and averaged over the two replicates, the mean for the Douglas-fir form was 64 ± 6% and was 9.8 ± 1.1% for the redcedar form ($p = 0.05$, TABLE II).

Comparison of Douglas-fir, redcedar, and Asian form haploids using ELISA. -All Douglas-fir form and Asian form haploids were visually rated as positive when reacted with DfAbxaR. The six redcedar form haploid isolates were each rated as negative when reacted with this antibody. In each replicate, the average ODs for the Douglas-fir and Asian isolates were similar, and those of the redcedar were statistically different ($p = 0.05$, TABLE III). The standardized mean of the three replicates was 28 ± 4.6% for the redcedar isolates, 80 ± 8.1% for the Douglas-fir isolates, and 87 ± 11.1 % for the Asian isolates (TABLE III). The latter two means were significantly different from that of the redcedar isolates ($p = 0.0001$).

The reaction of DfAb with the haploid isolates was not different enough to rate visually. The ranges of the ODs for the reaction of DfAb with the redcedar, Douglas-fir, and Asian forms were 0.100 to 0.185, 0.192 to 0.296, and 0.271 to 0.390, respectively. The corresponding mean ODs for these reactions were 0.139 ± 0.035,0.255 ± 0.042, and 0.340 ± 0.045, respectively. All three means were statistically different ($p < 0.005$).

[1]The use of trade or firm names is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

TABLE II

COMPARISON OF THE REACTIVITY OF REDCEDAR AND DOUGLAS-FIR FORM ISOLATES OF *PHELLINUS WEIRII* WITH A REDCEDAR CROSS-ABSORBED ANTIBODY MADE TO A DOUGLAS-FIR FORM ISOLATE IN ELISA

	Form	
	Redcedar	Douglas-fir
Number of isolates	26	29
OD range replicate 1	0.019–0.088	0.134–0.431
Mean OD replicate 1	0.045 ± 0.006	0.280 ± 0.03
OD range replicate 2	0.008–0.055	0.113–0.365
Mean OD replicate 2	0.034 ± 0.004	0.232 ± 0.02
Standardized range[a]	4.3%–20%	31%–100%
Standardized mean	9.8%	64%
95% confidence interval[b]	(48%, 61%)	

[a] Obtained by combining the two replicates after dividing each optical density (OD) by the OD of the homologous reaction in each replicate.
[b] For difference between two means.

Comparison of Douglas-fir and redcedar forms of P. weirii *using Western blots.* - DfAb binds to several minor protein bands (106, 42, 34, and 32 kDa) as well as to a major protein band at 57 kDa on Western blots of Douglas-fir isolates. This antibody binds to a single minor protein band at 57 kDa on blots of redcedar isolates (FIG. 1).

Comparison of haploid isolates from Asian, redcedar, and Dougklas-fir forms of P. weirii *using Western blots.* - DfAb binds to a major protein band at approximately 57 kDa on Western blots of haploid isolates of Douglas-fir and Asian form isolates and minor bands of the same size in redcedar isolates (FIG. 2). The same antibody also binds to a minor protein band at 63 kDa in isolates of all three forms. In addition, the antibody reveals common minor protein bands (31, 36, 40, 41, 51, and 55 kDa) in Douglas-fir and Asian form isolates.

DISCUSSION

The Douglas-fir and redcedar forms of *P. weirii* in North America are serologically distinct in ELISA when tested with an antibody made to the Douglas-fir form (DfAb). The two forms are statistically separable based on the ODs of the reaction products at 630 nm with DfAb. When DfAb is cross-absorbed with a redcedar isolate prior to use in ELISA, almost all cross-reactivity with the redcedar form is eliminated. The remaining activity is very specific for Douglas-lit' form isolates, which can be visually identified by the intensity of the reaction products in the microtiter wells. Much of the specificity possessed

TABLE III

REACTIVITY OF REDCEDAR, DOUGLAS-FIR, AND ASIAN FORM HAPLOID ISOLATES OF *PHELLINUS* WITH A REDCEDAR CROSS-ABSORBED ANTIBODY MADE TO A DOUGLAS-FIR FORM ISOLATE IN ELISA

	Form		
	Redcedar	Douglas-fir	Asian
Number of isolates	6	6	6
OD range replicate 1	0.039–0.076	0.114–0.216	0.166–0.280
Mean replicate 1	0.059 ± 0.014	0.175 ± 0.044	0.236 ± 0.046
OD range replicate 2	0.057 + 0.125	0.184–0.282	0.168–0.250
Mean replicate 2	0.088 ± 0.024	0.227 ± 0.045	0.221 ± 0.039
OD range replicate 3	0.041–0.142	0.204–0.335	0.222–0.369
Mean replicate 3	0.092 ± 0.049	0.291 ± 0.066	0.272 ± 0.058
Standaridzed range[a]	11%–44%	53%–100%	55% ± 127%
Standardized mean	28% ± 4.6%	80% ± 8.1%	87% ± 11.1%

[a] Obtained by combining the three replicates after converting the optical density (OD) from each reaction to a percentage of the homologous reaction in each replicate.

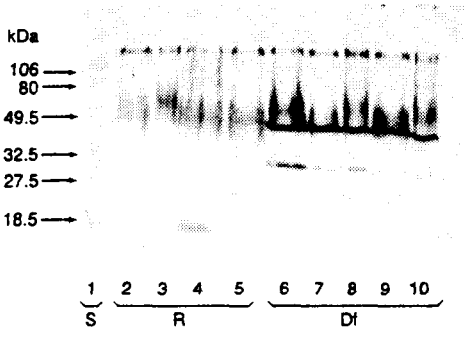

FIG. 1. Western blot of SDS-PAGE of four redcedar (lanes 2–5) and five Douglas-fir (lanes 6–10) form isolates of *Phellinus weirii* probed with an antibody prepared to a Douglas-fir form isolate. Lane 1 contains prestained molecular weight standards.

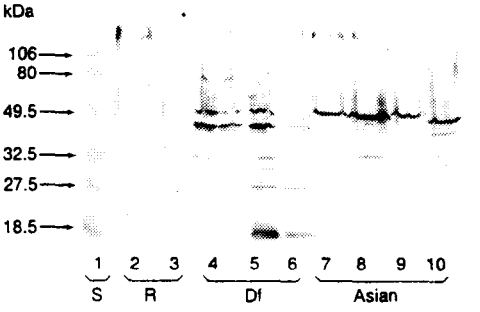

FIG. 2. Western blot of SDS-PAGE of two redcedar (lanes 1–2), three Douglas-fir (lanes 4–6), and four Asian (lanes 7–10) form haploid isolates of *Phellinus weirii* probed with an antibody prepared to a Douglas-fir form isolate. Lane 1 contains prestained molecular weight standards.

by DfAb may be explained by its reaction with a major protein band at 57 kDa identified in Western blots of isolates from Douglas-fir. This reaction is very weak in blots of the redcedar form isolates. However, the Douglas-fir form possesses several other antigenically active sites that distinguish it from the redcedar form.

In ELISA tests, DfAb is specific to both heterokaryotic and haploid isolates of the Douglas-tir form. DfAbxaR exhibits no reactivity with redcedar form haploid isolates, but is reactive with Dougias-fir and Asian form haploids. Western blots of all the haploid isolates probed with DfAb show a protein band at 57 kDa, as is the case for the heterokaryotic isolates. In Douglas-fir and Asian form isolates, this is a major band in the redcedar haploid isolates, it is a minor band. The Douglas-fir and Asian isolates also have numerous minor protein bands in common.

Antibodies were produced to three different redcedar form isolates, but data from experiments with these antibodies are not included here. None of these antibodies exhibited specificity for redcedar isolates in ELISA tests or Western blots. In fact. the Douglas-fir isolates reacted somewhat more strongly with the redcedar antibodies (RAb) than did the redcedar isolates. When attempts were made to remove the Douglas-fir reactive portion of the antibodies by cross-absorbing with a Douglas-fir form antigen, a slight specificity for redcedar form isolates was observed. This specificity was only evident statistically and was not usable for the identification of redcedar isolates. RAb also could not differentiate between the haploid isolates from the three forms in ELISA or Western blots. Western blots probed with this antibody possessed bands at 89 and 112 kDa, but no bands unique to redcedar form isolates were observed, Apparently, the redcedar isolates do not contain unique antigenic material in sufficient quantity to elicit the production of specific antibodies with the techniques employed.

Based on the reactivity of DfAb, it appears that the Douglas-fir and Asian forms are more closely related to each other than they are to the redcedar form. This result is in contrast to Angwin's **(1989)** conclusion that the two North American forms are more closely related to each other than they are to the Asian form. However, the isolates he used were from Japan and may be a different form than were the Siberian isolates employed for the serological data presented here.

The serological relatedness of the Douglas-fir form of *P. weirii* and the Siberian isolates supports Kotlaba and Pouzar's **(1970)** contention that Asian *P. sulphurascens* and *P. weirii sensu lato* are nonspecific. The Asian collections they examined were also from Siberia. Most North American specimens they used were collected from western redcedar and thus were presumably the redcedar form. However, serologically this form is less closely related to the Asian isolates than are the Douglas-fir isolates. This discrepancy may be the result of morphological similarities between the two North American forms, making their separation diflicult using Kotlaba and Pouzar's criteria. In addition, note that in this study only two Asian isolates were used and these may not be representative.

In conclusion, the data support the recognition of the Douglas-fir form and the redcedar form of *Phellinus weirii* in North America as separate species. The serological differences between these forms are substantial, thus allowing rapid identification of isolates using ELISA. The results also indicate that the Douglas-fir form of *P. weirii* is closely related to and probably nonspecific with the Asian species *P. sulphurascens* as suggested by Kotlaba and Pouzar **(1970).** Further studies of basidiomata, cultures, and DNA are needed to confirm our results.

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