Fatty acid composition of *Phellinus weirii* and *Polyporus versicolor* isolates

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Abstract

Eight isolates of *Phellinus weirii* and one *Polyporus versicolor* were grown in synthetic liquid medium. The mycelium was harvested and extracted to determine fatty acid content. Palmitic, stearic, oleic, and linoleic acids were detected in all *Phellinus* isolates; *P. versicolor* additionally contained myristic and linolenic acids. The major fatty acid in both fungi was linoleic acid, which comprised 68–84% of the total fatty acids. Two *Phellinus* isolates differed significantly in the level of linoleic acid; the other isolates did not significantly differ from each other in fatty acid patterns.

Introduction

Phellinus weirii (Murr.) Gilb. is a basidiomycete that causes a widespread and highly destructive root disease on conifer roots in northwestern United States and southern British Columbia. The fungus consists of innumerable ecotypes that in culture differ more or less in growth rate, appearance, sporulating capacity, and other characters. This study was designed to learn whether visibly different isolates also differ in lipid composition. Many fungi have been found to differ distinctively between species in lipid accumulation (Melhuish and Hacskaylo, 1975; Tyrrel, 1967, 1968, 1971). *Polyporus versicolor* L. ex Fries was also included for comparison of a white-rot saprophyte.

Materials and methods

Eight isolates of *Phellinus weirii* and one of *Polyporus versicolor* (Table 1) have been maintained in culture on identical media for more than 5 years. Each isolate was transferred from malt agar to 50 ml of synthetic liquid medium (Trione, 1964), modified by substituting 20 g sucrose with 10 g glucose in 250 ml Erlenmeyer flasks, and grown in still culture for 4 weeks at 25°C. Three flasks of each isolate were cultured. After incubation, mycelia were harvested by filtration and thoroughly washed with distilled water. Excess water in mycelia was removed by blotting with dry filter paper. Lipids were extracted immediately by the procedure of Folch *et al.* (1957), with chloroform:methanol (2:1, v/v), and the amounts present determined as solvent-free weight of the extract and expressed as percent to fresh mycelial weight.

Fatty acid methyl esters were prepared by heating the total lipids in a water bath for 15 min with 10% boron trichloride in methanol (Watson and Kramer, 1974). The esters were extracted into hexane and purified by thin-layer chromatography on silica gel G; hexane:diethyl ether (19:1) was the developing solvent. The plates were sprayed with 0.2% solution of 2′,7′-dichlorofluorescein in 95% ethanol. The esters, appearing as a yellow band under ultraviolet light (366 nm), were collected from the plates and eluted with diethyl ether. The diethyl ether was removed under nitrogen, and the residue was dissolved in 0.5 ml hexane.

Table 1 Sources of fungus isolates for fatty acid determination

Isolate	Host	Year of isolation	Geographic location
Phellinus weirii			
T-55	*Pseudotsuga menziesii* (Mirb.) Franco	1959	Gifford Pinchot National Forest near Randle, Washington
T-102	*P. menziesii*	1961	Clemons Tree Farm near Elma, Washington
T-124	*P. menziesii*	1961	Olympic National Forest, Washington
WL-2E	*Tsuga mertensiana* (Bong.) Carr.	1974	Waldo Lake, Oregon
WL-9	*T. mertensiana*	1973	Waldo Lake, Oregon
WL-NE10	*T. mertensiana*	1974	Waldo Lake, Oregon
WL-12	*T. mertensiana*	1973	Waldo Lake, Oregon
WL-13	*T. mertensiana*	1973	Waldo Lake, Oregon
Polyporus versicolor			
4937	Unknown	Unknown	Osaka, Japan

The methyl esters were analysed with a Microtek GC 2000-R gas chromatograph equipped with hydrogen flame detectors. A stainless steel column, 1.8 m x 2.1 mm, packed with 15% stabilized diethylene glycol succinate on 80- to 100-mesh chromosorb W, was operated isothermally at 200°C with a helium carrier gas flow rate of 40 ml/min. Inlet and detector temperatures were adjusted to 225°C. A column of less polarity containing 10% SP-2330 on the same support and operating at the same conditions was also used. Peaks were identified by comparison of the relative retention time of the standards and by a plot of the logarithm of retention time against carbon number. Fatty acids were quantified by measuring the peak height of each and determining its concentration from standard curves previously constructed through measurement of the peak height produced.

The results were analysed as a completely random design with the 8 df's for isolates partitioned into 1 df for contrasting *Polyporus* with eight isolates of *Phellinus* and 7 df's for discriminating among the eight *Phellinus* sources. Further, contrasts among the sources of *Phellinus* were made by Scheffé tests.

Results and discussion

The dry weight of mycelium varied with isolates. Most isolates grew well, producing an average 0.5–2.0 g mycelium per flask. Total lipid content differed significantly between some *Phellinus* isolates and ranged between 0.4% and 2.2% (Table 2). Average lipid content in *P. weirii* and *P. versicolor*, however, did not differ significantly.

The major fatty acids in all of the total lipid extracts of *P. weirii* were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) as shown in Figure 1. *Polyporus versicolor* additionally contained myristic acid (14:0) and linolenic acid (18:3) (Table 2, Figure 2). The most abundant fatty acid was linoleic acid, which comprised over 75% of the total fatty acid of all *Phellinus* isolates, significantly more than the 68% of the *P. versicolor* isolate. The two species also differed significantly in levels of stearic acid, oleic acid, and linoleic acid.

Among the eight *P. weirii* isolates, amounts of palmitic acid and linoleic acid differed significantly. The only significant difference detectable from Scheffé tests between individual means, however, was that the linoleic acid content of isolate T-102 was greater than that of WL-12.

Table 2 Mycelial weight, total lipids, and fatty acid composition of *Phellinus weirii* and *Polyporus versicolor*

Isolate	Mycelial wt (mg)	Total* lipids (% wt of mycelium)	Fatty acid, % of total, by weight*: 14:0	16:0	18:0	18:1	18:2	18:3
P. weirii								
T-55	464	2.1f	–**	16.7a	0.9a	1.3a	81.1ab	–
T-102	1517	0.4a	–	15.2a	0.4a	0.5a	84.0b	–
T-124	994	0.6bc	–	18.1a	1.1a	1.3a	79.5ab	–
WL-2E	896	0.5ab	–	17.1a	0.9a	1.1a	80.8ab	–
WL-9	1602	1.1e	–	19.4a	0.7a	0.5a	79.4ab	–
WL-NE10	735	0.8d	–	18.8a	0.7a	1.0a	79.4ab	–
WL-12	1977	0.7cd	–	21.2a	1.2a	0.8a	76.8a	–
WL-13	678	2.2f	–	17.3a	1.1a	1.3a	80.3ab	–
P. versicolor	579	1.1e	0.4	19.6a	1.4b	9.9b	68.2c	0.5

* Means within columns not sharing a common postscript differ significantly at the 0.05% confidence level with the Scheffé test.
** Fatty acid not detected.

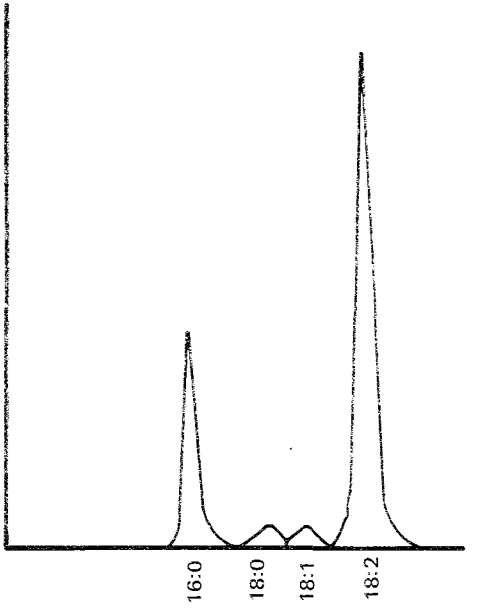

Figure 1 Gas chromatograms of mycelial fatty acids of *Phellinus weirii*.

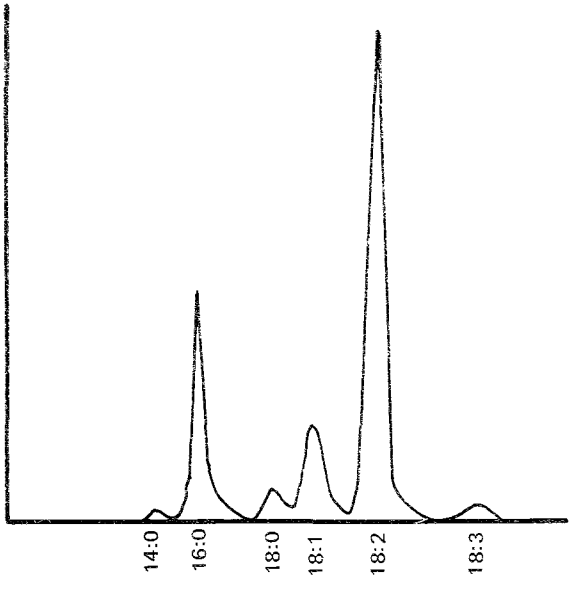

Figure 2 Gas chromatograms of mycelial fatty acids of *Polyporus versicolor*.

All these fatty acids exist mainly in bound forms in living fungal tissues. Large amounts of free fatty acids occur only from autolysis of cellular lipids following irreversible damage to tissues (Christie, 1973; Hitchcock and Nichols, 1971). Linoleic acid, a *P. weirii*-inhibiting fatty acid in red alder (*Alnus rubra* Bong.) as previously reported (Li *et al.*, 1970), would thus exist in free form in *P. weirii* in very small amounts or not at all.

The lipids of most fungi are comprised of 16:0, 18:0, 18:1, 18:2, and small or trace amounts of 14:0 or 18:3 (Jack, 1966). The results of this study indicate that *P. weirii*, as other basidiomycetes (Jack, 1966; Mehuish and Hacskaylo, 1975; Yokokawa, 1980), contained linoleic acid as a predominant fatty acid of mycelial lipids. In conjunction with other culture characters or mycelial protein patterns as reported by Li (1981), fatty acid composition can be a useful means of differentiating between *P. weirii* isolates. Such information is needed in determining the history of infection centres in the forest.

References

CHRISTIE W. W. 1973. *Lipid Analysis.* Pergamon Press, New York. 338 p.

FOLCH J., Lees J. and Sloane-Stanley G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226** 497–509.

HITCHCOCK C. and Nichols B. W. 1971. *Plant Lipid Biochemistry.* Academic Press, New York. 387 p.

JACK C. M. 1966. Lipid patterns in the major classes of fungi. *J. Bact.* **91** 2101–2.

LI C. Y., Lu K. C., Trappe J. M. and Bollen W. B. 1970. Inhibition of *Poria weirii* and *Fomes annosus* by linoleic acid. *For. Sci.* **16** 329–30.

LI C. Y. 1981. Phenoloxidase and peroxidase activity in zone lines of *Phellinus weirii*. *Mycologia* **73** in press.

MELHUISH J. H. Jr and Hacskaylo E. 1975. Fatty acid composition of ectomycorrhizal fungi *in vitro*. *Mycologia* **67** 952–60.

TRIONE E. J. 1964. Isolation and *in vitro* culture of the wheat bunt fungi *Tilletia caries* and *T. controversa*. *Phytopathology* **54** 592–6.

TYRREL D. 1967. The fatty acid composition of 17 *Entomorphthora* isolates. *Can. J. Microbiol.* **13** 755–60.

TYRREL D. 1968. The fatty acid composition of some Entomorphthoraceae. II. The occurrence of branched-chain fatty acids in *Conidiobolus denaesporus* Drechsl. *Lipids* **3** 386–72.

TYRREL D. 1971. The fatty acid composition of some Entomorphthoraceae. III. *Can. J. Microbiol.* **17** 1115–8.

WATSON J. L. and Kramer C. L. 1974. Fatty acid ratios of lipid solvent fractions in *Taphrina*. *Mycologia* **66** 773–9.

YOKOKAWA H. 1980. Fatty acid and sterol composition in mushrooms of ten species of polyporaceae. *Phytochemistry* **19** 2615–8.

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