Lipid Metabolism of *Agaricus bisporus* (Lange) Sing.: I. Analysis of Sporophore and Mycelial Lipids[1]

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ABSTRACT

The lipid components of four strains of *Agaricus bisporus* (Lange) Sing., the cultivated mushroom, were analyzed. Both sporophore and mycelial samples were obtained from beds in normal production. A method for obtaining mycelium free of compost was developed. Neutral lipids were separated from polar lipids by silicic acid column chromatography. Each fraction was separated by thin layer chromatography. Fatty acid methyl esters were analyzed by gas liquid chromatography and mass spectrometry. Sporophore extracts contained free sterol, free fatty acid, triglycerides, phosphatidyl choline and phosphatidyl ethanolamine. High amounts of linoleic acid were found in both neutral and polar lipid fractions. Mycelial extracts contained free fatty acids, triglycerides, phosphatidylcholine and phosphatidyl ethanolamine. No free sterol could be detected. Linoleic acid was also present in large amounts.

INTRODUCTION

A preliminary analysis of the lipid components of the sporophore of *Agaricus bisporus* (Lange) Sing., the cultivated mushroom, was conducted by Hughes (1). He reported a large amount of free sterol, smaller quantities of sterol ester, triglyceride, free fatty acids and phospholipids. Ten fatty acids were identified and linoleic acid was found to account for 63% to 74% of the amount of fatty acids present, depending on the mushroom variety. Maggioni et al. (2) reported that the total lipid fraction of sporophores growing on nitrogen supplemented compost contained 71% linoleic acid and 15% palmitic acid.

It has been shown that mushroom production increased when lipid material was added to compost (3), and that mushroom mycelial growth was increased when lipids were added to basal nutrient media (4). To better understand lipid metabolism in *A. bisporus*, a more detailed analysis of the lipid components was felt desirable and the present study was initiated. Lipid components of both sporophore and mycelium were analyzed. Neutral and polar lipids were separated. Strains 310 (white), 314 (off white), 320 (brown) and 322 (off white) were compared. Strains 310, 320 and 322 are used in commercial spawn making and account for at least 70% of the mushrooms produced in the United States and Canada. Strain 314 was included as it was the strain which failed to respond with increased mycelial growth to lipid addition.

MATERIALS AND METHODS

Isolation of Sporophore and Mycelial Lipids

Strains 310, 314, 320 and 322 were obtained from the culture collection of The Pennsylvania State University. The compost was prepared as described by Schisler (3) without the addition of any supplementary nutrients at casing. Mycelial samples were harvested by a special method designed to get mycelium free from compost material. Compost was filled into wooden trays (61 x 61 x 14 cm). When filled the trays contained 25-28 kg of compost. Trays were filled with compost and spawned (seeded with mushroom mycelium) to one half their depth. A layer of fiberglass fly screen was placed on top of the compost. A 1 cm layer of water saturated Superlite (Perlite Products, Primos, Pa.) was spread on top of the screening and covered by a second piece of screening. The tray was then filled and spawned to its capacity. The screens were removed from the compost after three weeks. The Superlite clinging to the screen due to the mycelial growth was scraped off into flasks and extracted. A portion of the same compost was spawned for sporophore harvest. After three weeks spawn growth the trays were cased (covered with a layer of topsoil to induce fruiting). Mushrooms were harvested from the first fruiting. Lipids in both sporophore and mycelial samples were extracted according to the method of Folch et al. (5). The neutral lipid fraction was separated from the polar lipid fraction using a silicic acid column as described by Hirsch and Ahrens (6).

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TABLE III

Fatty Acid Composition of the Neutral and Polar Lipids of Mycelium[a]

Acid	Strain 310	Strain 314	Strain 320	Strain 322
Neutral lipids				
C10:0	4.1	t	12.1	t
C12:0	5.2	14.6	6.2	7.8
C14:0	8.3	2.9	t	5.2
C16:0	22.1	25.0	19.6	27.6
C16:1	6.6	4.3	9.8	6.1
C17:0	t	10.4	9.0	9.0
C18:0	10.0	7.5	6.7	8.4
C18:1	16.9	18.7	18.6	16.2
C18:2	26.3	16.2	17.6	19.4
Polar lipids				
C10:0	t	t	t	t
C12:0	t	2.0	2.4	1.8
C14:0	t	6.8	5.1	2.4
C16:0	18.8	16.7	18.0	15.2
C16:1	8.5	8.8	6.7	3.7
C17:0	5.1	4.7	3.8	4.5
C18:0	3.7	4.5	2.8	5.5
C18:1	14.1	13.7	12.5	11.1
C18:2	49.8	42.4	48.4	55.3

[a]Values are expressed as per cent of total. Those values marked (t) are less than 1% of the total.

high proportion of linoleic acid. Shaw found that the polar lipid contained most of the linoleic acid.

The results of this study confirmed those of Shaw; however, the advantage of the study reported here was that a valid comparison of mycelial and sporophore lipids of a single species was possible. Sporophores and mycelia were harvested in normal production under a controlled environment and not in vitro on artificial media. It was not necessary to correlate data from diverse species to compare mycelial to sporophore lipids.

Bentley et al. (11) reported that the ratio of neutral lipid to polar lipid in the fruiting body of *Clitocybe illudens* was 1:2 and that the neutral fraction was primarily free sterol. It can be seen in Table I that of the four strains of *A. bisporus* used in this study only strain 310 had a similar ratio of neutral to polar lipids in the fruiting bodies. Free sterol was, however, the predominant lipid in the sporophore neutral fraction.

Utilization of lipid by *A. bisporus* has been shown to be a key factor in the nutrition of the commercial mushroom. Schisler (3) showed that lipid supplementation to compost resulted in increased sporophore yield. Wardle and Schisler (4) showed that lipid additions to basal media gave increased mycelial growth. Ethyl esters of oleic and linoleic acids were shown to be primary stimulatory components.

It is interesting to speculate on the implications of some of the differences in lipid composition of the mycelium and sporophore as found in this study on the lipids of *A. bisporus*. Distinct differences in the weight per cent of the neutral and polar lipids between the sporophore and the mycelium were shown (Table I). The absence of sterol in the mycelium and its appearance in quantity in the sporophore suggests that free sterol is the end product of synthetic events during the fruiting process.

The fatty acids of the sporophore extracts from the various strains were consistent in profile, with the exception of 314 which was significantly lower in linoleic acid than the other three strains. This low level of linoleic acid was concomitant with the fact that strain 314 was giving a low crop yield at the time of sampling. Wardle and Schisler (4) also reported that the growth of mycelium of strain 314 was not increased by ethyl-linoleate supplement. The inability of this strain to utilize linoleic acid may be a cause for low yield. Minor differences in fatty acid composition between strains found in this study are probably attributable to the differences in the metabolism of each strain.

REFERENCES

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2. Maggioni, A., C. Passera, F. Renosto and E. Benetti, J. Agr. Food Chem. 10:517 (1968).
3. Schisler, L.C., Appl. Microbiol. 15:844 (1967).

TABLE II

Fatty Acid Composition of the Neutral and Polar Lipids of Sporophores[a]

Acid	Strain 310	Strain 314	Strain 320	Strain 322
Neutral lipids				
C10:0	t	---	---	---
C12:0	t	t	t	t
C14:0	t	4.9	t	t
C16:0	13.2	26.5	16.3	15.4
C16:1	t	t	t	t
C18:0	4.1	7.4	6.7	2.5
C18:1	5.4	12.2	8.9	3.3
C18:2	77.1	49.0	68.0	78.6
Polar Lipids				
C10:0	t	---	---	---
C12:0	t	t	t	t
C14:0	t	t	t	t
C16:0	6.1	9.2	6.5	5.7
C16:1	t	t	---	---
C18:0	2.0	2.3	1.4	1.8
C18:1	t	t	---	t
C18:2	91.3	88.5	92.0	92.4

[a]Values are expressed as per cent of total. Those values marked (t) are less than 1% of the total.

The ratio of neutral to polar lipids was higher in the mycelium than the sporophore.

A thin layer separation of sporophore neutral lipids showed that free sterol and monoglycerides were the major lipid classes. Smaller amounts of free fatty acids and triglycerides were observed (Fig. 1-A). The areas with Rf values equal to triglycerides, free fatty acids and monoglycerides were scraped from a thin layer separation of neutral lipids. IR spectra of the eluates were similar to reference standard compounds. Upon saponification and esterification of the triglyceride and monoglyceride fractions, fatty acid methyl ester patterns similar to those of the neutral lipids were established by gas chromatography.

Mycelial neutral lipids contained free fatty acids, triglycerides and monoglycerides. No free sterol was detected (Fig. 1-B).

Phosphatidyl choline and phosphatidyl ethanolamine were the predominant polar lipid classes (Fig. 2). The compounds had similar Rf values on two-dimensional thin layer chromatography (TLC) as phosphatidyl choline and phosphatidyl ethanolamine from bovine milk polar lipids and authentic reference compounds. Both compounds reacted with the Dittmer-Lester spray. The compound with an Rf value equal to phosphatidyl ethanolamine reacted with ninhydrin. The IR spectra of the two compounds were superimposable on those of authentic phosphatidyl choline and phosphatidyl ethanolamine. Small amounts of phosphatidyl serine and phosphatidyl inositol were tentatively identified in sporophore polar lipids having similar Rf values as authentic reference compounds and of those components in bovine milk polar lipids. Both reacted with the Dittmer-Lester spray. Neither of these compounds was detectable in mycelial polar lipids. However, the mycelial polar lipids had distribution of phosphatidyl choline and phosphatidyl ethanolamine identical to the sporophore polar lipids.

A typical percentage composition of fatty acids in sporophore neutral and polar lipids was tabulated (Table II). Palmitic, stearic, oleic and linoleic acids accounted for over 90% of the fatty acids present. In the neutral lipids of sporophores, linoleic acid accounted for approximately 70% of the fatty acids. Strain 314, however, had only 49% linoleic acid. Mycelial neutral lipids showed more varied fatty acid compositions than did sporophore neutral lipids (Table III).

The polar lipid extracts of both sporophore and mycelium were predominantly palmitic and linoleic acid (Tables II and III). The sporophore polar fraction was high in linoleic acid, averaging about 90%, whereas the mycelial polar lipids average about 50% of this acid.

DISCUSSION

The lipid components of several basidiomycetes were investigated by Shaw (10). He collected sporophores growing in the wild and reported that fruiting bodies and mycelia had a

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