Mushroom Mycelium from Submerged Culture Offers Possible Food and Pharmaceutical Products

MUSHROOM MYCELIUM

Experiments with Submerged Culture

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The possibility of growing mushroom mycelium in submerged culture was investigated because of the large quantities of low-cost potential growth media available in Florida in the form of citrus press water. The mycelium of *Agaricus blazei* (M) has been grown in submerged culture on orange juice, citrus press water, and synthetic media. Nutritionally, the mycelium compares favorably with some food sources rich in amino acids and B vitamins. The mycelium, when prepared as a food, lacks the true mushroom flavor; however, because of its bland taste it might be useful for pharmaceutical concentrates of B vitamins and amino acids.

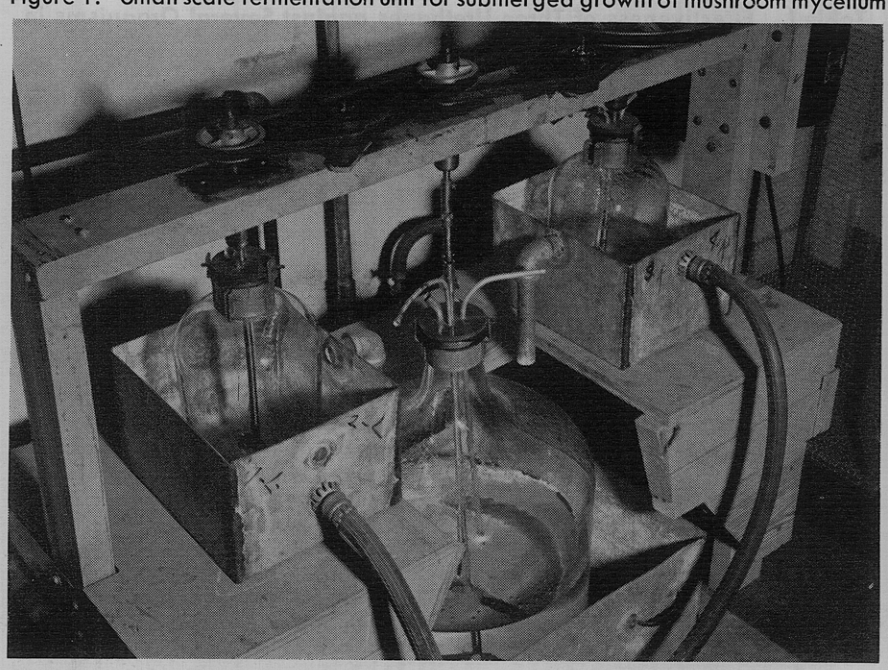
THE MUSHROOM INDUSTRY in the United States has grown rapidly since its infancy at the beginning of the 20th century. Although mushrooms themselves have not acquired the popularity in this country that they enjoy in Europe, where they have been eaten for centuries, soups and sauces containing mushrooms are consumed in great quantities.

Mushroom propagation as practiced today is an agricultural operation that has changed very little from the process described by Duggar (*4*) in 1904. In 1948, Humfeld (*6*) announced a process which offered the promise of large-scale, low-cost production of mushroom mycelium on an industrial basis. The mycelium, rather than the fruiting body, is produced (*6–8*) by submerged culture in aerated liquid media, similar to the method of propagation of molds for antibiotic production. Szuecs (*19*) reported a process for growth of mushroom mycelium and the preparation of an extract or concentrate having the characteristic flavor and taste of the edible mushroom, *Agaricus campestris*. The economic possibility of these processes was of particular interest to the authors because of the large quantities of low-cost potential growth media available in Florida in the form of citrus press water, an inedible liquid that contains the residual juice after squeezing and the fluid extract from fresh citrus peel and pulp. If used in the production of a food material, this press water could be handled to meet sanitary requirements.

Cultivation of Mycelium

Humfeld (*8*) and Szuecs (*19*) reported the use of isolated strains of the commercial cultivated mushroom, *Agaricus campestris*. The authors used a related wild mushroom, *Agaricus blazei* (*12*), an excellent edible species, which is native to

Figure 1. Small scale fermentation unit for submerged growth of mushroom mycelium



Florida and grows at a higher temperature than *A. campestris*. In most microbiological processes employing submerged culture considerable heat is evolved, and if the organism will withstand higher temperatures less cooling is required. A pure culture of *Agaricus blazei* was obtained by aseptically transferring pieces of the mushroom tissue onto 2% malt agar slants. The mycelium grown on these slants was employed as inoculum for the liquid submerged culture media. The liquid cultures were grown in 250-ml. Erlenmeyer flasks which were agitated on a reciprocating shaker having a 2-inch stroke set at 80 cycles per minute. For larger batches, the mycelium was grown in 1-liter or 40-liter bottles with stirring and aeration (Figure 1). A liquid medium that was employed had the following composition: 5% glucose, 0.2% potassium nitrate, 0.1% potassium dihydrogen phosphate, 0.05% magnesium sulfate heptahydrate, 0.05% calcium nitrate, 0.01% sodium chloride, and a trace of ferrous sulfate. The mycelium was separated from the broth by filtration or centrifugation.

In preliminary experiments the mycelium grew in ball-like clumps in the aerated liquid culture medium. The size of the balls varied, depending on the type of agitation, aeration, and the sugar concentration of the medium. In shaker flasks the balls were about 0.125 inch in diameter and looked like fish eggs, while in aerated bottles the clumps were as large as 1 inch in diameter but much less dense than the smaller clumps. In one of the shaker runs it was noted that the mycelium in one of the flasks was finely dispersed, giving a milky suspension. When this was cultured on rye grains, abortive fruiting bodies like those of the parent strain were formed, demonstrating that the culture was related to *A. blazei*. Microscopically, the mycelium was finer than that of the original culture (Figures 2 and 3). The culture contained numerous spore-like bodies (Figure 4) similar to the "secondary spores" described by Kligman (*10*) and Humfeld (7). The milky appearance and rapid growth of this culture in liquid media appear to be the result of the germination and growth of the "secondary spores." After about 48 hours the mycelial strands begin to fragment and at 72 hours the fragments are very abundant in the culture (Figure 5). These fragments also contribute to an increased growth, as each individual fragment serves as a nucleus for new growth.

This new strain, *A. blazei* (M), undoubtedly developed as a mutation from the parent strain. It has been employed in these studies on the submerged culture growth of this mushroom because it is better adapted to growth in liquid media, giving a more rapid rate of growth and higher yields of mycelium than the parent strain.

Humfeld and Sugihara (*9*) have given an excellent report of the physiology and nutrition of *Agaricus campestris*. *Agaricus blazei* differs from *A. campestris* in its physiology, as demonstrated by inability to grow and flourish in composted manure as does *A. campestris*. An investigation of the nitrogen requirements of *A. blazei* demonstrated that it utilized inorganic nitrogen as satisfactorily as organic nitrogen. Ammonium salts, nitrates, and urea gave excellent growth of the mycelium. It was possible to grow the mycelium on a synthetic medium of inorganic minerals with glucose as the only carbon source, but yields were not so high as on a diluted orange juice medium fortified with the same mineral salts. Both the synthetic and orange juice media were used extensively in these investigations.

Yield of Mycelium

Table I shows that the original pH of the medium had very little effect on the total yield of mycelium from synthetic medium. The mycelium buffered the medium at approximately pH 5.5, as noted by the range of final pH values. In orange juice medium, the results were different, in that the pH continued to rise to approximately pH 8.0, depending on the duration of the run (Table II). This gradual increase in

Table I. Effect of Initial pH on Yield

		Synthetic Medium			
pH		Sugar utilized, % (initial sugar 5%)	Mycelium produced, g./100 ml. dry weight	Efficiency, % (Fermentation)[a]	Plant[b]
Initial	Final				
3.5	5.5	4.0	1.65	41	33
4.8	5.5	4.5	1.86	41	37
5.5	5.4	3.5	1.52	42	30
6.5	5.9	4.5	1.78	40	36
7.5	5.6	4.5	1.74	39	35

[a] Based on sugar fermented.
[b] Based on initial sugar.

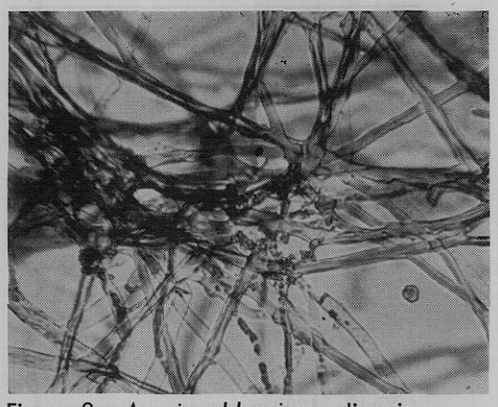


Figure 2. *Agaricus blazei* mycelium in liquid medium after 36 hours (970X)

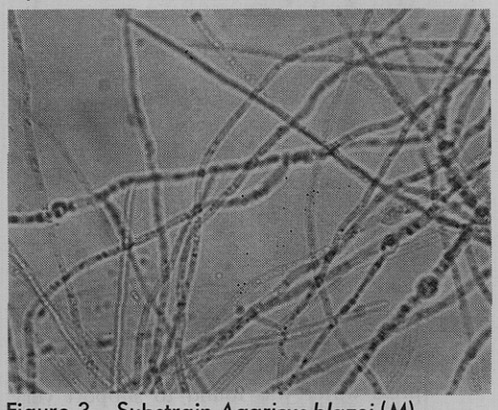


Figure 3. Substrain *Agaricus blazei* (M) in liquid medium after 36 hours (970X)
Note the "secondary spore" which has germinated

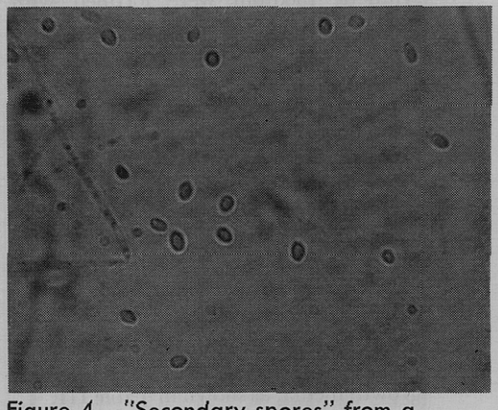


Figure 4. "Secondary spores" from a culture of *Agaricus blazei* (M) (970X)

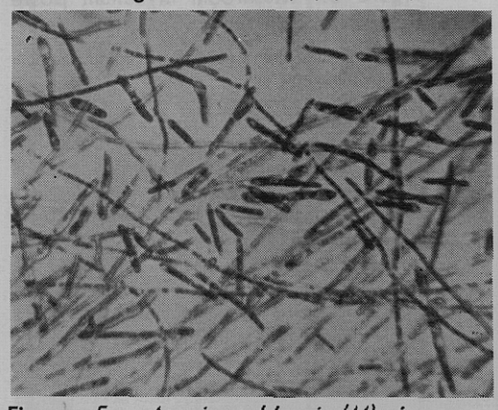


Figure 5. *Agaricus blazei* (M) in liquid medium after 72 hours showing presence of the mycelial fragments (970X)

Table II. Twenty-Liter Run on Orange Juice Medium

Days	Sugar Remaining	Cell Volume, Ml. Cells/10 Ml.	pH
0	5.6	...	4.55
1	5.5	0.3	4.70
2	5.4	0.5	4.95
3	4.8	0.9	5.40
4	3.5	1.3	6.00
6	2.0	1.5	7.22
7	2.0	1.5	7.35
8	1.8	1.5	7.55
9	1.8	1.5	7.80

Yield. 1200 grams wet, 214 grams dry
Efficiency. Fermentation 31%, plant 20%

pH probably resulted from the utilization of citric acid in the juice. Table II presents the results of a small pilot plant fermentation on orange juice medium. After 6 days there was no further increase in cell volume, but even after 9 days there was still a residual 2% sugar which had not been utilized. The mycelium harvested from this run contained about 85% moisture and was a pasty, cream-colored product very similar to compressed yeast (Figure 6).

In preliminary experiments, citrus press water was found to support very poor growth of the mycelium. This was a result of toxicity rather than improper nutrition, for both the press water and the synthetic medium were fortified with mineral nutrients. Dilution of the toxic press water with sufficient synthetic medium permitted satisfactory growth. The toxic factors themselves have not been detected, but they were found to arise in the storage of the press water. Fresh press water was not toxic, but press water stored in the refrigerator for several weeks would not support growth. By freezing the press water, it was possible to store it without change. The fresh citrus press water proved to be a satisfactory growth medium and compared favorably with other well-known growth media, as shown in Table III.

Figure 6. Mushroom mycelium paste (left) from pilot run

Flask contains the mycelium in liquid medium before filtration.

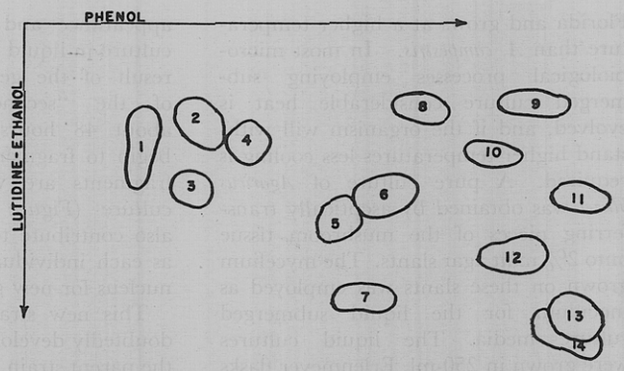


Figure 7. Chromatogram for amino acids in *Agaricus blazei* fruiting body

Amino Acid and B Vitamin Content of Mycelium

Mushrooms are a source of proteins and B vitamins. The protein content of the commercial mushroom on a dry weight basis has been reported as 37.5% (*1*). Humfeld (*8*) reported the mushroom mycelium of his white variety to be 35.5% protein, while the brown variety was 45.3%. The fruiting body of *A. blazei* growing wild has been found to be 43% protein, whereas the mycelium had only 32.5% protein. The protein content of the mycelium, however, may merely be a reflection of the nitrogen concentration of the medium. The figures given above were all determined on the basis of a protein containing 16% nitrogen. If the value of 11.79% nitrogen determined by Fitzpatrick *et al.* for purified mushroom protein (*5*) is taken, the protein content is 1.36 times as great.

Although the amino acid content of *A. campestris* sporophores has been studied (*2, 5, 21*), there is nothing in the literature on the amino acids of mushroom mycelium. A qualitative identification was made of the amino acids of the mycelium of *A. blazei* (M) and of the fruiting bodies of *A. blazei* and *A. campestris* by two-dimensional paper chromatography (*17*). The solvent systems, reported by Block (*3*), were phenol-water and 2,6-lutidine–ethyl alcohol–water. The chromatographs were run on Whatman No. 1 filter paper and were developed with ninhydrin by heating at 100° C. for 30 seconds. Amino acids were identified by R_f values as compared to those of known amino acids. Table IV lists the amino acids identified on the chromatograms.

A protein hydrolyzate of the mycelium of *A. blazei* (M) and the fruiting body of *A. blazei* showed the presence of 14 amino acids and the possible presence of two more. Methionine, leucine, and isoleucine could not be separated on the chromatograms; therefore the presence of only one of these could be assured. The experimental method did not test for cystine or cysteine; therefore these amino acids were not accounted for. The similarity of the amino acid content of the mycelium of *A. blazei* (M) and the fruiting body of *A. blazei* may be seen by comparison of Figures 7 and 8. In the analysis of the fruiting body of the commercial mushroom, *A. campestris*, serine and proline were found to be absent (Figure 9). They are not, however, essential amino acids. Tryptophan was present in the fruiting bodies of *A. blazei* and *A. campestris* and in the mycelium of *A. blazei* (M), as determined by the Hopkins-Cole test on the alkaline hydrolyzates. Qualitatively, the amino acid content of *A. blazei* (M) compares favorably with that of other common proteinaceous food materials.

The B vitamin content of *A. blazei* (M) mycelium was determined (*11*), employing standard microbiological methods (*13–16*). A comparison of B vitamins in the mushroom mycelium and other foods which are sources of the B vitamins is given in Table V. Compared to the edible portion of the whole wheat kernel, the mushroom and its mycelium are rich in the B vitamins. The mycelium of *A. blazei* (M), a white mushroom, was not greatly different in B vitamin content from the mycelium of the *A. campestris* (white variety) of Humfeld. The mushroom mycelium might serve as a source of B vitamins for the production of vitamin concentrate preparations. It is probable that the

Table III. Mycelium Production on Different Media

Medium	Sugar Utilized, % (Initial Sugar 6%)	Mycelium Produced, G./100 Ml. Dry Weight	Efficiency, %	
			Fermentation	Plant
Citrus press water	4.3	1.88	46	31
Orange juice	5.0	2.06	41	34
Malt extract	3.9	1.98	51	33
Nutrient broth	5.3	2.28	43	38
Corn steep liquor	5.2	2.66	51	44

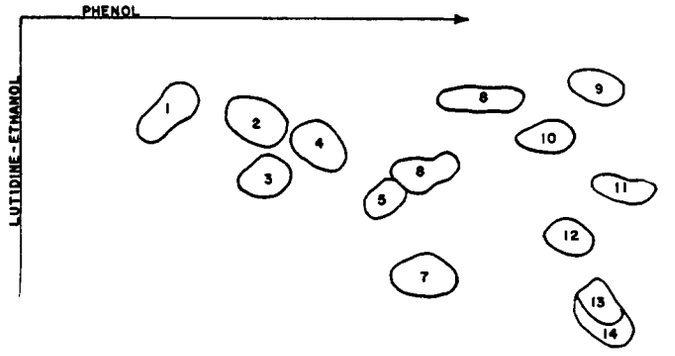


Figure 8. Chromatogram for amino acids in *Agaricus blazei* (*M*) mycelium grown in submerged culture

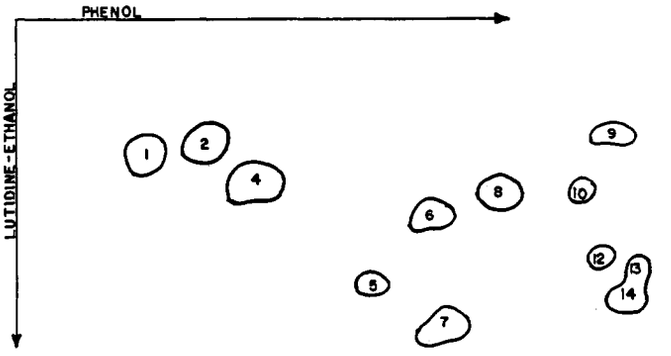


Figure 9. Chromatogram for amino acids in *Agaricus campestris* fruiting body

quantity of vitamins produced in the mycelium could be increased by studying the effect of variations in composition of media and conditions of growth.

Flavor

When the mushroom mycelium from synthetic medium was fried or toasted, it had little or no flavor. The mycelium grown on orange juice medium had a bitter taste which, however, could be removed by washing with water. When the mycelium was grown on other media, it had some of the taste of the substrate on which it was grown. When the mycelium from the synthetic medium was heated gradually to about 75° C., the cells were autolyzed, as shown by the decrease in viscosity of the mycelium paste. This treatment brought out the flavor of the mycelium, but this flavor in no way resembled the flavor of a fresh, cooked mushroom. Mushroom soups made with this mycelium were appetizing and had a flavor something like that of commercial canned mushroom soups. The texture of the soups prepared with the mycelium differed from that of the commercial soups, because of the lack of pieces of mushroom in the soup. The texture of fresh mushrooms can be simulated to some degree by pressing mycelium into pieces and permitting these pieces to knit together by the subsequent growth of the mycelium. Nevertheless, the production of a true mushroom flavor remains the primary problem before commercial production is practicable. In appraising the flavor of their *A. campestris* mycelium, Humfeld and Sugihara (*9*) conservatively state "..... the flavor is designated as mushroomlike pending more extensive tests." Fundamental questions that remain to be answered are whether the true mushroom flavor is a product of only the specialized cells of the fruiting body and cannot be produced by the mycelium, or whether precursors normally found in soil or manure but not in the laboratory media are required for synthesis of the flavoring compounds in the mycelium.

Table IV. Amino Acid Key for Figures

1.	Aspartic acid	9.	Arginine
2.	Glutamic acid	10.	Histidine
3.	Serine	11.	Proline
4.	Glycine	12.	Valine
5.	Threonine	13.	Methionine,
6.	Alanine		leucine, and
7.	Tyrosine		isoleucine[a]
8.	Lysine	14.	Phenylalanine

[a] Not separated by technique used; therefore presence of only one could be assured.

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Table V. B Vitamins of Mushroom Mycelium, Commercial Mushrooms, Yeast, and Wheat

(Mg./100 grams dry weight)

	Mushroom Mycelium			Commercial Mushroom			
	A. blazei	*A. campestris*		(*A. campestris*)	Torula Yeast	Brewer's Yeast	Wheat
Vitamin	(*M*) (*7*)	White (*5*)	Brown (*5*)	(*1*)	(*16*)	(*17*)	(*17*)
Thiamine	0.2	0.9	0.9	1.2	0.7–4.2	10	0.58
Riboflavin	3.4	4.7	9.0	5.2	2.4–4.7	5.8	0.16
Niacin	14.6	19.0	29.0	58.0	37–69	39	4.8
Pantothenic acid	6.9	...	...	23.0	10–18	...	...