Adaptability to submerged culture and amino acid contents of certain fleshy fungi common in Finland

Marja Liisa Hattula and *H. G. Gyllenberg*
Department of Microbiology, University of Helsinki, Finland

The literature on the possibilities of submerged culture of macrofungi is already voluminous. However, the interest has merely been restricted to the traditionally most valued fungi, particularly members of the genera *Agaricus* and *Morchella* (HUMFELD, 1948, 1952, HUMFELD & SUGIHARA, 1949, 1952, SUGIHARA & HUMFELD, 1954, SZUECS 1956, LITCHFIELD, 1964, 1967 a, b, LITCHFIELD & al., 1963). The information obtainable from studies on these organisms is not especially encouraging because the rate of biomass production seems to be too low to compete favourably with other alternatives of single cell protein production (LITCHFIELD, 1967 a, b). On the other hand, the traditional use of fungi as human food all over the world is a significant reason for continued research on the submerged production of fungal mycelium. If the fungal mycelium is comparable with other single-cell protein preparations as to its protein content and amino acid composition, it seems to gain preference over other alternatives due man's traditional knowledge and experience concerning the toxicity characteristics and other conditions influencing the edibility of fungi. A particular aspect requires attention in this connection. The traditional «mushroom« used as food concerns the fruiting bodies whereas the submerged cultivation produces preparations of mycelium. Therefore it seems that besides the adaptability to submerged growth the similarity in composition of the mycelial preparation to the corresponding fruiting bodies must be considered when the suitability of fungi for submerged production is evaluated. The present work concerns a collection of fungi common in Finland which have been investigated according to the criteria described above.

MATERIAL

The material consisted of 33 species of fungi, 29 of which were obtained from the Department of Silviculture, University of Helsinki, through the courtesy of Professor P. Mikola and Mr. O. Laiho. Four species were isolated by the present authors by taking a sterile piece of the fruiting body at the point where the cap and the stem are joined and by transferring it on a slant of suitable agar. The species considered are listed below following the nomenclature of M. MOSER (1967). (For the species indicated by an asterisk fruiting bodies were collected for comparison whereas the species with the + label were isolated in pure culture by the present authors.)

Clavaria ligula
Cantharellus cibarius *
Suillus grevillei (elegans)
S. luteus *
S. bovinus *
S. variegatus *
Boletus edulis * +
Leccinum testaceo-scabrum * +

L. sp. («Boletus versipellis«)
L. scabrum *
Paxillus incolutus *
P. atrotomentosus
Hygrophoropsis aurantiaca («Cantharellus a.«)
Tricholomopsis rutilans (Tricholoma r.)
Tricholoma flavobrunneum
T. imbricatum
Lyophyllum loricatum
Armillariella mellea (Armillaria m.) *
Collybia dryophila *
C. butyracea
Micromphale perforans (Marasmius p.)
Marasmius androsaceus
Macrolepiota procera (Lepiota p.)
Agaricus arvensis * +
Cystoderma amianthinum
Coprinus comatus * +
Hypholoma capnoides
Kuehneromyces mutabilis (Pholiota m.)
Cortinarius hemitrichus
Lactarius repraesentaneus *
L. necator *
L. deliciosus aggr. *
L. rufus *

The pure cultures were maintained on Modess' agar the composition of which is the following (Modess, 1941): KH_2PO_4 0.5 g, $MgSO_4 \cdot 7\ H_2O$ 0,5 g, NH_4Cl 0,5 g, Malt extract 5 g, Glucose 5 g, $FeCl_3$, 1 % sol. 0.5 ml, Agar. 15 g, Distilled water 1000 ml. The medium was sterilized at 116°C for 20 minutes.

The mycelia were inoculated on new agar slants once a month and the tubes were kept four weeks at room temperature (22—24°C) after which they were transferred to refrigerator temperature. For sixteen of the test organisms (indicated by in the above list) fruiting bodies were collected. These were cleaned of dust and sand by scraping and no water was used for cleaning. The fruiting bodies were cut into small pieces and dried at 40°C until they were easy to grind to fine powder. The powder was kept in a closed wessel in the refrigerator.

RESULTS

Adaptability to submerged culture

The adaptability to submerged culture was investigated by some experiments where the mycelia were grown on different media. The primary criterion in these experiments was how rapidly the fungi used the sugar in the medium. The first screening experiment to omit the most slowly growing species was made by growing the mycelia in the three following media:

a) 5 % malt extract,
b) Modess' medium (Modess 1941),
c) Reusser's medium (Reusser & al., 1958) the composition of which is as follows:

Glucose 40 g, Ammonium tartrate 8 g, KH_2PO_4 0.5 g, $MgSo_4 \cdot 7\ H_2O$ 0,5 g, $CaCl_2$ 50 mg, $FeSO_4 \cdot 7\ H_2O$ 2 mg, $MnSO_3 \cdot H_2O$ 2 mg, Pyridoxine 1 mg, p-Aminobenzoic acid 1 mg, Thiamine 0.5 mg, Distilled water 1000 ml. The glucose was sterilized separately and the vitamins were added in separate solution which was sterilized by filtration. All three media were bottled in 250 ml Erlenmeyer-flasks, 80 ml in each, and they were sterilized at 116°C for 20 minutes.

The inoculation was made by transferring two agar slants of every species into 80 ml 5 % malt extract. The flasks were incubated 10 days at room temperature as stationary cultures. The grown mycelium was washed with sterile distilled water and was then broken by use of a sterile Ultra-Turrax-machine. The homogenized mycelium was suspended into 80 ml of sterile water and 10 ml of this suspension was used to inoculate each flask. Because of the different amount of growth of the test organisms the suspensions employed as inocula were made visually as similar in density as possible. Every organism and medium was tested in duplicate. The flasks were incubated for two weeks in a rotary shaker (200 rpm) at 28°C. After this period the growth was scored visually. Table 1 shows the results of the experiment.

On the basis of the results of the experiment indicated in Table 1, the following species were omitted from further study: *Paxillus involutus, P. atrotomentosus, Tricholoma flavobrunneum, Tricholomopsis rutilans, Lactarius repraesentaneus, L. deliciosus, Marasmius perforans, M. androsaceus, Cystoderma amianthinum, Boletus edulis* and *Boletus «versipellis«*.

An experiment concerning the sugar consumption was made with the remaining species in order to evaluate the utilization of carbon and the ylield of mycelium per quantity of sugar used. Reusser's medium, which contains glucose, and Modess' medium, which contains maltose in addition to glucose, were

Table 1. Submerged growth of fungi in different media. +++ growth excellent, ++ good growth, + weak grofth, — no growth (the amount mycelium equal to the inocolum).

Species	5 % malt extract	Modess' medium	Reusser's medium
Clavaria ligula	++	+++	+++
Cantharellus cibarius	+++	+++	+++
Suillus grevillei	++	++	++
Suillus luteus	++	+	++
Suillus bovinus	+++	++	+++
Suillus variegatus	+	+	+
Boletus edulis	—	+	+
Leccinum testaceo-scabrum	+++	+++	+++
«*Boletus versipellis*«	—	—	++
Leccinum scabrum	—	++	++
Paxillus involutus	+	+	+
Paxillus atrotomentosus	—	—	—
Hygrophoropsis aurantiaca	++	+	+
Tricholomopsis rutilans	—	—+	++
Tricholoma flavobrunneum	—	+	—
Tricholoma imbricatum	—	++	—
Lyophyllum loricatum	+	+	+
Armillariella mellea	+++	+++	+++
Collybia dryophila	++	+	+++
Collybia butyracea	++	++	++
Micromphale perforans	+	+	+
Marasmius androsaceus	+	+	+
Macrolepiota procera	++	++	++
Agaricus arvensis	+	+	+
Cystoderma amianthinum	++	—	—
Coprinus comatus	+	+	+
Hypholoma capnoides	++	++	+
Kuehneromyces mutabilis	+	+	+
Cortinarius hemitrichus	—	++	+++
Lactarius repraesentaneus	—	—	—
Lactarius necator	++	++	++
Lactarius deliciosus aggr.	—	—	—
Lactarius rufus	—	+	++

chosen for this experiment. Sugar determinations were made (a) before inoculation and (b) at the time the incubation was interrupted occording to the method of Bertrand (Grossfeld, 1935). A two weeks old stationary culture of mycelium was used as inoculum. The mycelium was broken by Ultra-Turrax into sterile water and 10 ml of

Table 2. The efficiency of mycelium production, gram of mycelium/100 grams of sugar.

Mushroom	Reusser's medium	Modess' medium
Cantharellus cibarius	*54.6*	*46.7*
Suillus grevillei	*42.7*	*42.2*
Suillus luteus	12.9	24.3
Suillus bovinus	*53.3*	22.6
Suillus variegatus	*42.4*	16.9
Leccinum testaceo-scabrum	*36.4*	*36.2*
Leccinum scabrum	1.5	3.7
Hygrophoropsis aurantiaca	*26.8*	*33.5*
Tricholoma imbricatum	1.0	5.4
Lyophyllum loricatum	*48.7*	*36.7*
Armillariella mellea	*43.8*	*32.4*
Collybia dryophila	8.8	24.5
Collybia butyracea	1.3	8.2
Macrolepiota procera	—	12.1
Agaricus arvensis	1.8	6.9
Coprinus comatus	0.8	2.6
Hypholoma capnoides	3.9	4.8
Kuehneromyces mutabilis	6.0	*28.0*
Cortinarius hemitrichus	*57.4*	*50.2*
Lactarius necator	4.5	13.4
Lactarius rufus	0.7	2.5
Clavaria ligula	1.0	1.9

the suspension was inoculated into 80 ml of the medium in a 250 ml Erlenmeyer flask. The flasks were incubated in a rotary shaker (200 rpm) at 28°C. Incubation was continued until a considerable amount of rapidly growing mycelium had developed. During the incubation the volume of the medium was controlled by adding distilled water to compensate for evaporation losses. The mycelium was separated by Büchner-filtration, and was then dried to constant weight. Table 2 shows the efficiency of submerged growth expressed as grams of mycelium per 100 grams reducing sugar.

The results clearly indicated that several of the species included in this experiment grow too slowly to be useful for submerged mycelium production. The growth rate seemed to depend on the medium as well as on the species. However, both the media employed are commonly used in the submerged cultivation of fungi. In either or both of the media the following ten species yielded mycelium to an amount higher than 25 % of the weight of the utilized sugar and might thus be worth further attention: *Cantharellus cibarius, Hyprophoropsis aurantiaca, Suillus*

bovinus, S. variegatus, S. grevillei, Leccinum testaceoscabrum, Armillariella mellea, Lyophyllum loricatum, Kuehneromyces mutabilis and *Cortinarius hemitrichus.*

Amino acid content

For the production of mycelium for amino acid analysis the fungi were grown in submerged culture in 2 liter fermentors which contained 1 liter medium. The temperature was 28°C, agitation 280 rpm and the aeration rate was 1 l air/min/l medium. Although the first experiments had shown that Reusser's medium would not be optimal for all the test organisms this medium was used in these experiments because its composition is exactly defined. The first cultivations were made with the species which had the greatest yield coefficients in the sugar consumpiton experiments. In addition mycelia of those species for which fruiting bodies were available were also produced.

The inocula were made from two weeks old stationary cultures of the corresponding fungi. The mycelia were washed with water, broken with Ultra-Turrax and suspended in sterile water. These suspensions were used as inocula. The inocula were made visually as similar in density as possible. The growth was followed by taking twice a week a sample for sugar and ammonium nitrogen determinations (GROSSFELD 1935, CONWAY 1957). The cultivation was considered finished when all the sugar was consumed. The grown mycelium was separated by Büchner filtration and dried at room temperature. The dry mycelium was ground to fine powder and the determinations of moisture and ash content (A.O.A.C. 13 004, 13 006, 1960) as well as of nitrogen content (WILSON & WILSON, 1960) were made from the powder. The total protein content was calculated by multiplication of the nitrogen value obtained by the micro-Kjeldahl method by 6.25. Two species, *Collybia dryophila* and *Paxillus involutus* were cultivated in Erlenmeyer flasks in a rotary shaker because the culture in fermentors did not succeed due to contamination.

Table 3. Growth of the mycelia in submerged culture and comparison of the main constituents of the mycelium to the corresponding figures for the fruiting bodies.

Species	Growth days required to complete consumption of glucose	g mycelium/ 100 g reducing sugar	Mycelium		Fruiting bodies	
			ash % of dry mycelium	protein % of dry mycelium	ash % of dry material	protein % of dry material
Cantharellus cibarius	17	23.1	3.91	28.5	9.44	21.5
Suillus grevillei	16	20.3	6.35	36.4	—	—
Suillus luteus	20	62.0	4.25	23.8	5.85	16.4
Suillus bovinus	18	41.4	3.26	25.9	5.04	20.9
Suillus variegatus	35	15.9	3.67	30.3	5.36	9.8
Boletus edulis	50	57.9	4.98	37.6	7.66	33.8
Leccinum testaceo-scabrum	14	43.3	3.53	38.5	5.84	28.7
Leccinum scabrum	19	21.3	4.46	47.1	5.28	32.0
Paxillus involutus	grown in shaker		3.50	30.4	10.96	29.7
Hygrophoropsis aurantiaca	18	38.4	2.09	23.2	—	—
Lyophyllum loricatum	7	32.3	4.01	38.3	—	—
Armillariella mellea	17	42.1	8.97	18.9	8.66	21.0
Collybia dryophila	grown in shaker		1.95	22.1	8.81	30.4
Agaricus arvensis	53	20.8	3.97	42.4	11.24	55.1
Coprinus comatus	52	13.1	4.70	39.2	9.28	23.0
Kuehneromyces mutabilis	21	22.3	7.08	49.7	—	—
Cortinarius hemitrichus	17	31.8	3.74	40.9	—	—
Lactarius necator	49	45.9	2.81	21.4	8.82	22.3
Lactarius rufus	29	46.3	2.25	17.8	7.84	34.2

The growth of the mycelia is presented in Table 3 where the main composition of the mycelia is also compared with the corresponding data for the fruiting bodies. The results show that the growth rate of the fungi was very low. Compared with microorganisms grown in submerged culture even the shortest cultivation time with *Lyophyllum loricatum*, i.e. seven days, must be considered far too long. The average growth time in the medium used was 27 days. The average protein content in the mycelia (32.2 %) cannot be regarded as high but it was higher than in corresponding fruiting bodies (27.1 %).

The amino acid determinations were carried out in the following way: The dry powdered preparation of either mycelium or fruiting bodies was heated at 103°C to constant weight and 100 mg of this material was weighed into 100 ml of 6-n HCl. Nitrogen gas was bubbled through the solution before hydrolysis. The hydrolysis was made under reflux at 180°C and nitrogen was conducted into the vessel through the condenser. After hydrolysis the solution was filtered to remove humin and the hydrocloric acid was removed in Rotawapor evaporator at 40°C. Distilled water was added and evaporated until the pH of the solution was about 2. One drop of 30 % H_2O_2 and some ml:s of water were added to the dry amino acids to oxidize cysteine to cystine and the solution was allowed to stand for one hour at room temperature. The amino acids were diluted with 12.5 % sucrose solution, the pH was adjusted to 1.9—2.1 and the final volume of the solution was 25 ml, one ml of which was analyzed by Technicon Auto Analyzer.

There are notes in the literature on losses of amino acids during hydrolysis, but, on the other hand, according to a number of investigators valine will not be liberated at the same time with other amino acids. To obtain reliable results the mushroom material was hydrolyzed for different periods of time, 12, 20, 24 and 72 hours. After 12 hours hydrolysis only α-ε-diaminopimelic acid was released quantitatively. There was no evident difference in the amino acid composition of the material hydrolyzed for 20 and 24 hours, and the 20 hours hydrolysis was chosen as the zero time. No rise in the valine content occured after 72 hours hydrolysis even if a metal catalyst was used, but

Table 4. Amino acid composition of Leccinum scabrum, Cantharellus cibarius and Armillariella mellea and the average composition of the amino acids of the mycelium and the fruiting body preparations investigated. Amino acids are given as grams per 16 gram protein nitrogen.

Amino acid	*Leccinum scabrum*		*Cantharellus cibarius*		*Armillariella mellea*		Amino acids average	
	fr. body	myc.	fr. body	myc.	fr. body	myc.	fr. body	myc.
Aspartic acid	6.88	6.89	7.04	8.32	10.09	6.52	7.30	7.19
Threonine	3.81	3.51	3.21	5.27	4.70	4.33	4.02	4.28
Serine	3.59	3.58	3.40	4.14	5.70	3.99	3.92	4.07
Glutamic acid	11.88	11.47	10.24	10.73	14.52	7.51	11.03	9.65
Proline	3.27	3.55	2.79	3.76	3.84	2.79	3.70	3.80
Glycine	5.55	3.94	3.24	4.33	3.96	2.96	4.27	3.85
Alanine	6.31	5.78	3.85	5.57	2.83	4.05	4.62	4.94
Valine	0.27	0.23	0.63	1.57	0.36	0.53	0.53	0.31
Cystine	4.39	4.93	3.56	5.04	5.17	3.98	4.41	4.82
Methionine	0.30	0.88	0.80	1.00	—	—	0.24	0.34
α-ε-diaminopimelic acid	18.83	9.76	15.85	12.61	11.86	10.47	14.70	13.43
Isoleucine	2.51	7.36	5.42	5.47	4.65	5.74	3.32	5.08
Leucine	3.57	6.11	4.96	6.67	5.57	5.18	4.85	5.89
Tyrosine	1.36	2.85	1.95	2.79	2.76	2.19	2.51	2.73
Phenylalanine	2.87	3.46	2.67	3.02	1.76	2.75	2.60	3.10
γ-aminobutyric asid	0.59	0.07	0.56	0.60	0.78	2.82	1.59	1.13
Ammonia $(NH_4)_2SO_4$	11.12	10.36	13.54	8.13	10.02	18.86	12.63	10.62
Ornithine	0.93	0.09	3.93	0.38	0.40	0.40	0.64	0.30
Lysine	3.68	4.31	3.55	5.34	4.44	4.43	3.88	4.80
Tryptophan	1.46	1.63	1.28	3.12	1.71	3.24	1.79	2.19
Histidine	2.58	2.61	2.26	2.62	1.91	2.23	2.49	2.54
Arginine	4.73	6.19	5.20	4.87	5.31	5.05	5.04	5.01

Table 5. The range of variation in the amounts of amino acids in the protein of different fungi compared to the FAO recommendations, amino acids g/16 g protein nitrogen.

Amino acid	Fruiting body	Mycelium	FAO recommendations
Aspartic acid	6.06—10.09	2.66— 9.92	
Threonine	2.07— 5.23	1.83— 5.32	
Serine	2.57— 5.10	2.10— 5.24	
Glutamic acid	6.52—15.31	3.41—13.72	
Pyroline	2.76— 5.44	2.69— 5.75	
Glycine	3.32— 5.55	1.96— 4.70	
Alanine	2.83— 6.31	3.82— 5.78	
Valine	0 — 0.80	0 — 1.57	4.2
Cystine	3.47— 5.78	1.66— 5.87	
Methionine	0 — 0.80	0 — 1.08	2.2
α—ε-*diamino-pimelic acid*	6.93—21.21	5.05—54.35	
Isoleucine	0 — 6.39	0 —14.13	4.2
Leucine	0 — 8.82	2.01— 8.09	4.8
Tyrosine	0 — 4.62	1.12— 3.86	
Phenylalanine	0 — 3.64	0 — 3.86	
γ-*aminobutyric acid*	0.33— 4.69	0.07— 2.67	
Ammonia	2.18—26.26	5.95—15.95	
Ornithine	0 — 3.93	0 — 1.09	
Tryptophan	1.32— 3.17	1.24— 3.72	1.4
Lysine	3.31— 6.14	1.35— 8.39	4.2
Histidine	1.91— 3.44	0.91— 3.44	
Arginine	3.31— 6.77	1.61— 8.39	

the amounts of cystine and isoleucine increased and the amount of increase was constant. The amount of aumonia atso increased as a result of the destrunction of labile amino acids. After 72 hours hydrolysis losses of aspartic acid, threonine and serine occured. In the final results all the values are extrapolated to the zero time. Because of the complete destruction of tryptophan during the acid hydrolysis it was determined separately employing the enzymatic method described by SPIES (1967), which is based on the use of pronase (Calbiochem). The selected methods were maintained constant throughout all the analyses and every determination of amino acids was made immediately after hydrolysis to avoid losses which occur in solutions.

Because it seems purposeless to describe in detail the amino acid composition of 18 different mycelia and 14 preparations of fruiting bodies, three species are chosen to give a representative picture of the amino acid composition. The fruiting bodies of these species are commonly used as food and the experience of their adaptation to submerged cultivation has been satisfactory in the authors' experiments. The amino acid composition of the fruiting bodies and the mycelia of *Leccinum scabrum, Cantharellus cibarius* and *Armillariella mellea* and the average amino acid composition of the fungi investigated are presented in Table 4 (a corresponding comparison of the amino acid contents of the mycelium and fruiting bodies of *Boletus luteus* is presented in the accompanying paper; HATTULA & GYLLENBERG, 1969). The range of variation in the amounts of amino acids considering all the fungi investigated as compared to the FAO recommendations are presented in Table 5.

The present results show that the amino acid composition in different species is very similar in the mycelium and the fruiting body, respectively. The main component is ninhydrinpositive α-ε-diaminopimelic acid which is not known to be of nutritional value. All the essential amino acids are present in most of the species but the amounts of valine and methionine are very small. The small amonut of methionine in microbial protein is well known but the small amount of valine seems to be characteristic of Finnish fungi. There are, however, very few species of fungi in which other essential amino acids than methionine and/or valine are lacking. The fruiting body of *Lactarius necator* contains no valine, methionine, isoleucine and phenylalanine but in the corresponding mycelium only methionine is lacking. There occurs in the fruiting body of this fungus more than 50 % of α—ε-diaminopimelic acid as compared with the total amount of amino acids. If the average contents of the essential amino acids are compared to the FAO recommendations only valine and methionine remain both in the mycelia and in the fruiting bodies below the limits defined. The amount of cystine in the Finnish fungi is unusually high. Because cystine can substitute for methionine up to 80 % in the diet the low valine content seems to constitute the only real amino acid deficiency of the fungi investigated.

SUMMARY

Out of 33 species of fungi only ten were found to adapt easily to submerged growth. However, these organisms, too, required as a rule 14 day or more for a complete utilization of 4 per cent sugar in a synthetic medium. The highest yield coefficient for mycelium production (g mycelium per 100 g consumed sugar) among these fungi was 57 *(Cortinarius hemitrichus)* whereas the highest protein content in dry mycelium was 49.7 % *(Kuehneromyces mutabilis)*. The protein content of the mycelia produced in submerged culture was on the average somewhat higher than the corresponding figure for the fruiting bodies of the same fungi. The amino acid composition of the protein was in general the same in corresponding preparations of mycelium and fruiting bodies. Except for valine and methionine fungal protein seems to be a satisfactory source of essential amino acids.

REFERENCES:

Association of Official Agricultural Chemists, 1960: Ninth edition A.O.A.C., P.O. Box B, Franklin Station, Washington 4 D.C., 158.

Conway, E. J., 1957: Microdiffusion analysis, 4th edition, Crosby Lockwood and Son Ltd., London, 465.

FAO Nutritional Studies No. 16, 1957: Food and Agricultural Organization of the United Nations, Rome.

Grossfeld, J., 1935: Kohlenhydrate in Handbuch der Lebensmittelchemie, ed. A. Böhmer, Springer Verlag, Berlin III, 2, 866—868.

Hattula, M. L. & H. G. Gyllenberg, 1969: Protein and fat composition and vitamin content of Boletus luteus mycelium produced in submerged culture. Karstenia 9, 46—50.

Humfeld, H., 1948: The production of mushroom mycelium (Agaricus campestris) in submerged culture, Science, 107, 373.

Humfeld, H., 1952: Production of mushroom mycelium, U.S. Patent 2 618 000.

Humfeld, H. & T. F. Sugihara, 1949: Mushroom mycelium production by submerged propagation, Food Technol., 3, 355—356.

Humfeld, H. & T. F. Sugihara, 1952: The nutrient requirements of Agaricus campestris grown in submerged culture, Mycologia, 44, 605—620.

Litchfield, J. H.: 1967 a: Submerged culture of mushroom mycelium, Microbial Technology, Reinhold Publishing Corporation, New York, Amsterdam, London, 107—144.

Litchfield, J. H., 1967 b: Submerged culture of Mushroom mycelium, Food Technol., 21, 55—57.

Litchfield, J. H., R. C. Overbeck & R. S. Davidson, 1963: Factors affecting the growth of morel mushroom mycelium in submerged culture, Agr. Food Chem., 11, 158—162.

Modess, O., 1941: Zur Kenntniss der Mykorrhizabildner von Kiefer und Fichte, Sym.bot. upsal., V 1:1 16.

Moser, Meinhard, 1967: Die Röhrlinge und Blätterpilze (Agaricales). In: Helmut Gams: Kleine Kryptogamenflora, Band II b/2 (Basidiomyceten II. Teil). Gustav Fischer Verlag, Stuttgart 433 pp., 429 figs. in 13 pls.

Reusser, F., I. F. T. Spencer & H. R. Sallans, 1958: Protein and fat content of some mushrooms grown in submerged culture, Appl. Microbiol., 6: 1—4.

Spies, J. R., 1967: Determination of tryptophan in proteins, Anal. Chem., 39, 1412—1416.

Sugihara, T. F. & H. Humfeld, 1954: Submerged culture of the mycelium of various species of mushroom, Appl. Microbiol., 2, 170—172.

Szuecs, J., 1956: Mushroom culture, U.S. Patent 2 761 246.

Wilson, G. L. & D. W. Wilson, 1960: Comprehensive Analytical Chemistry, Elsevier Publishing Company, Amsterdam, London, New York, Princeton, 505.