Trans. Br. mycol. Soc. **86** (4), 519–525 (1986) *Printed in Great Britain*

PROTEIN UTILIZATION BY BASIDIOMYCETE FUNGI

BY H. M. KALISZ, D. MOORE

Department of Botany, The University, Manchester M13 9PL

AND D. A. WOOD

Glasshouse Crops Research Institute, Worthing Road, Littlehampton, West Sussex BN17 6LP

Three species of basidiomycete fungi, *Agaricus bisporus*, *Coprinus cinereus* and *Volvariella volvacea*, were grown on defined liquid media under conditions of proteinase induction (in the presence of protein as sole source of carbon, nitrogen or sulphur) and derepression (in the absence of any source of one of these major elements). All three species utilized protein as sole source of carbon, nitrogen and sulphur. Protein was utilized as efficiently as was glucose when provided as a sole source of carbon. When supplied together as carbon sources both protein and glucose were utilized more rapidly, and growth was greater, than when either protein or glucose were supplied separately as sole sources of carbon. Thus no catabolite repression was observed in the presence of glucose and, similarly, no catabolite repression was observed when media were supplemented with ammonium chloride as well as protein.

Agaricus bisporus (Lange) Pilát and *Volvariella volvacea* (Bull.: Fr.) Sing. are grown commercially for food production on composted wheat and rice straw, respectively. *Coprinus cinereus* (Schaef.: Fr.) Gray, the common weed fungus, is a frequent contaminant of mushroom beds. The nature of the nitrogen source(s) in compost is still unknown but protein is probably the most abundant nitrogen source available to these organisms in the form of lignoprotein, microbial protein and plant protein. Very little is known about how readily or efficiently these fungi can utilize protein molecules. Micro-organisms are also important in the growth of *A. bisporus* mycelium in compost (Eddy & Jacobs, 1976; Stanek, 1972; Fermor & Wood, 1981). The microbial biomass of compost probably serves as a concentrated source of nitrogen, carbon and minerals for the mushroom and may contribute significantly to its growth and yield. Microbial polysaccharides may also provide a source of water due to their high water-holding capacity (Wood & Fermor, 1985). *A. bisporus* is able to degrade dead bacteria and to utilize them as sole source of carbon, nitrogen and phosphorus (Fermor & Wood, 1981). However, microbial biomass contributes about 10% of the carbon nutrition of mushroom biomass (Sparling, Fermor & Wood, 1982).

The bulk of compost or straw consists of macromolecules, such as lignin, cellulose, hemicellulose and protein, as well as microbial biomass. Ligno-protein polymers constitute the largest fraction degraded during mycelial growth of *A. bisporus* in compost, and cellulose and hemicellulose during fruiting (Gerrits, 1969). These nutritional changes are correlated with changes in extracellular enzyme activities in the compost (Wood & Goodenough, 1977). *Coprinus* excretes large amounts of ammonium nitrogen into the medium, which implies an ability to utilize the carbon skeletons of amino acids (Stewart & Moore, 1974; Al-Gharawi & Moore, 1977).

Very little is known about the nutritional requirements and biochemistry, especially enzymology, of *V. volvacea*. Recent studies by Quimio (1981) have shown the organism to be capable of growth on a number of defined media, though more slowly than on natural media. The organism can utilize a number of carbon and nitrogen sources, with sucrose the best source of carbon and urea of nitrogen. Glutamic acid was shown to inhibit growth of *Volvariella* (Quimio, 1981).

The present study was undertaken to investigate the ability of the three basidiomycete species to utilize protein as a sole source of carbon, nitrogen and/or sulphur.

MATERIALS AND METHODS

Stock cultures of *Agaricus bisporus* strain D791 and of *Volvariella volvacea* Indonesia strain were maintained on 2% (w/v) malt extract agar (slopes and plates). Stocks of *Coprinus cinereus* Birmingham dikaryon strain (ATCC 42721) were maintained on complete medium agar. The cultures were incubated at 25 °C.

Preparation of inoculum

Cultures 7–10 day-old of *C. cinereus* and *V. volvacea*, and 3–4 week-old cultures of *A. bisporus*

Vol. 86, Part 3, was issued 26 April 1986

were transferred to liquid media prior to inoculum preparation. *C. cinereus* was grown on a defined basal medium (25 ml in a 250 ml flask), designated SNC (Stewart & Moore, 1974), supplemented with 1% (w/v) D glucose (BDH), 30 mM-NH_4Cl (Hopkins & Williams), and 1% (w/v) Bacto casamino acids (Difco). *A. bisporus* was grown on a basal medium, designated Treschow, as determined by Treschow (1944) and described by Fermor & Wood (1981). The medium was supplemented with 1% (w/v) glucose and 2 mM-L-glutamate (Sigma), and 50 ml was used in each 250 ml flask. *V. volvacea* was grown on a 2% Bacto malt extract (Difco) liquid medium (50 ml in a 250 ml flask). All the media were sterilized by autoclaving at 15 p.s.i. for 15 min. The three species were incubated in the dark at 25° in static culture.

Mycelial suspensions were prepared by centrifuging liquid cultures of the organisms on a bench centrifuge (5000 rev. min^{-1}, 20 min) in sterile centrifuge tubes. The supernatant was decanted, the pellet rinsed with sterile distilled water and resuspended in approximately the same volume of distilled water, containing about 100 glass beads (4·5–5·5 mm diam). The suspensions were shaken in an orbital shaker (300 rev. min^{-1}, 20 min) to provide a fragmented mycelial suspension. Volumes of 0·5–1·0 ml of each suspension were added to the test media.

Conditions of growth

Each of the species was grown on Treschow medium supplemented with one of twelve different combinations of: 1·0% (w/v) insoluble casein (BDH no. 44018); 1·0% (w/v) glucose; 30 mM-NH_4Cl; and/or 0·05% (w/v) $MgSO_4.7H_2O$ (in the absence of a sulphur source, the sulphate was replaced with equimolar $MgCl_2.6H_2O$). The different combinations used are listed below. Basal media solutions and nutrient solutions were autoclaved separately, subsequently mixed in suitable proportions, and made up to 25 ml volumes in 250 ml flasks. These were inoculated with 0·5 ml volumes of mycelial suspensions. All test media were prepared at least in duplicate, and mostly in triplicate. The cultures were incubated in static conditions at 25° in the dark. *Coprinus* cultures were harvested at 3-d intervals up to day 15. *Agaricus* cultures were harvested on day 9, and then every 8 d up to day 49. *Volvariella* cultures were harvested after 4 d, and then every 5 d up to 24 d. The cells were harvested by filtration using 7 cm diam Whatman GF/A filter disks. The filtrate was tested for the quantity of exogenous protein, ammonia, glucose and pH. Unless otherwise stated, each parameter from every sample was tested in triplicate.

Analytical methods

Dry weight determination. The mycelium, harvested by filtration, was dried to constant weight at 60°. Adjustments were made to account for insoluble casein retained by filtration in the early stages of the experiment.

Protein determination. Extracellular protein was determined by adding 0·25 or 0·5 ml of the medium to 1·0 ml of 10% TCA, and incubating overnight at 4°. Precipitated protein was recovered by centrifugation and assayed by the Lowry method. Protein concentration was determined against a casein standard.

Determination of culture filtrate glucose. Glucose was assayed using a glucose assay kit (Sigma no. 510), interfering substances being removed by precipitation with 0·15 M-$Ba(OH)_2$ and 5% (w/v) $ZnSO_4.7H_2O$.

Determination of ammonia. To 2·5 ml of medium was added 25 μl of 10 M-NaOH, after thorough mixing the amount of free ammonia released was determined using an Orion Research ammonia electrode.

RESULTS

Experiments were done with a total of twelve different medium constitutions. Eight of the mixtures contained protein (as 1% insoluble casein) and in one this was the sole source of C, N and S; the other seven had all possible combinations of single, double and triple supplementation with additional carbon (1% glucose), nitrogen (30 mM-NH_4Cl) or sulphur (0·05% $MgSO_4.7H_2O$). Of the remaining four mixtures which lacked protein supplementation, one contained all three major elements (as glucose, ammonium and sulphate) while each of the other three lacked one or other of these.

Growth yield

Protein and glucose were used about equally well as sole sources of carbon by *A. bisporus* D791 grown on Treschow medium, but growth of this strain (in terms of maximum mycelial dry weight yield) was 3–4 times greater in media containing both glucose and protein than in media containing only one of these supplements as carbon source (Fig. 1). In media containing both protein and glucose, addition of ammonium resulted in a 20% increase in maximum dry weight attained. However, when protein was included as sole source of carbon, the addition of ammonium affected both the maximum dry weight yield and the pattern of growth of

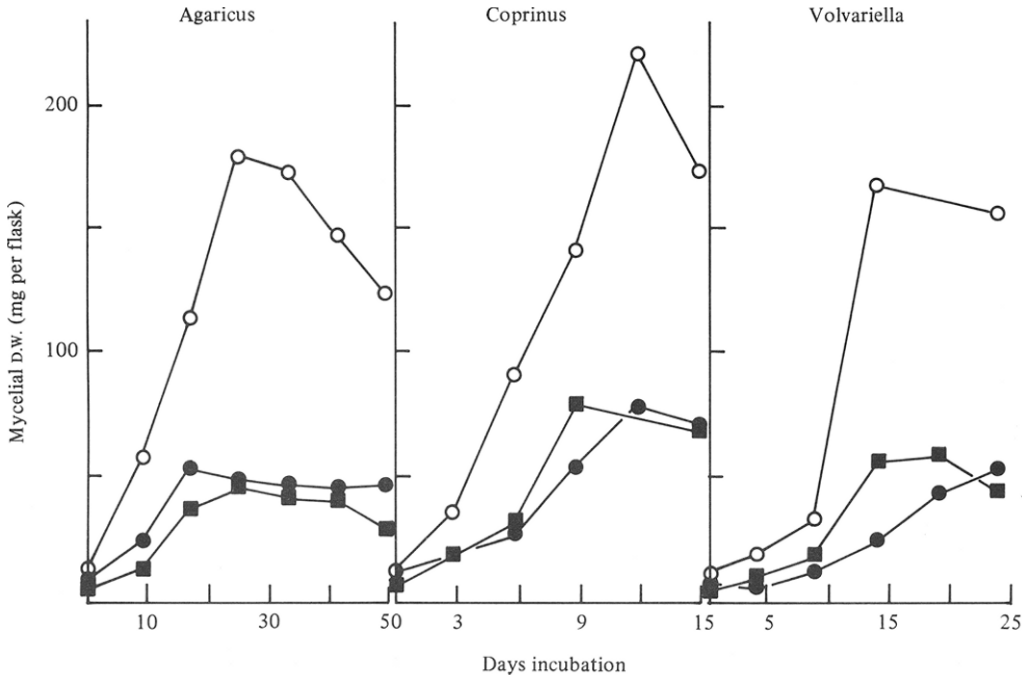

Fig. 1. Biomass yields of shake-flask cultures of three basidiomycetes grown in medium supplemented with protein + ammonium + glucose (○); protein + ammonium (●); or glucose + ammonium (■). Data points are the means of three replicates, standard error being less than 10% of the mean.

Agaricus. In media supplemented with ammonium, maximum weights of 50 mg per flask were attained on day 17; without ammonium supplementation, i.e. protein = sole source of carbon and nitrogen, mycelial dry weight increased gradually to reach a maximum of 39 mg per flask after 49 d incubation. Sulphate supplementation had no effect on growth yield.

Maximum dry weights of *C. cinereus* grown in the presence of protein were also increased 3–4-fold by additional glucose (Fig. 1). Additional ammonium and sulphate had little effect on dry weight yields. In media supplemented with either protein or glucose as sole sources of carbon about the same maximum mycelial dry weights (of about 80 mg per flask) were observed. However, the rate of biomass increase in media containing glucose was twice that observed in media containing protein as sole carbon source.

In media deficient in protein and sulphate, biomass yields of *Coprinus* were about 50% of those in media supplemented with all three major elements, suggesting a considerable ability to scavenge and reutilize sulphur from the inoculum. *A. bisporus* did not respond in this way.

Dry weight yields of *Volvariella* were increased as much as 7-fold on addition of glucose to Treschow medium supplemented with protein and in the presence of both carbon sources (protein and glucose), growth was enhanced by further supplementation with the ammonium salt. In Treschow medium without glucose (protein as sole source of carbon) maximum dry weight yields were observed on day 14, with additional ammonium effecting a 20% decrease in yield. The effect of sulphate addition was not tested.

Protein utilization

Protein utilization was assessed by measuring the amount of TCA-precipitable material in the medium. About 65% of the supplied protein was removed from the medium in the first 17 d incubation of *A. bisporus* cultures; and by the 49th day 15% remained (Table 1). This pattern was unaffected by supplementation with glucose, ammonium or sulphate. On the other hand additional glucose had a marked effect on the rate of protein utilization by *C. cinereus* (Table 1). In the presence of both glucose and protein, 90% of the protein was degraded in the first 6 d. In the absence of glucose, the 90% degradation level was reached on day 12. Additional ammonium and sulphate supplementation had only marginal effects on the rate of protein utilization by *C. cinereus*. In *Volvariella volvacea*

Table 1. *Amount of residual substrate protein (mg per flask) left in the medium during incubation of cultures of* Agaricus, Coprinus *and* Volvariella

Organism	Days growth	Supplements to the medium: Protein	Protein + glucose	Protein + ammonium	Protein + sulphate
Agaricus	0	205·8 ± 3·2 (2)	203·2 ± 0·6 (2)	201·1 ± 3·4 (2)	202·5 ± 8·5 (2)
	9	136·6 ± 6·1 (3)	163·4 ± 18·1 (3)	134·4 ± 7·0 (3)	141·3 ± 13·2 (3)
	17	67·5 ± 2·7 (3)	69·1 ± 2·9 (2)	64·9 ± 3·2 (2)	74·6 ± 3·4 (2)
	25	70·5 ± 0·5 (2)	35·3 ± 7·0 (3)	55·4 ± 3·7 (3)	67·9 ± 3·3 (3)
	33	49·9 ± 0·5 (3)	26·0 ± 1·2 (3)	37·4 ± 8·8 (3)	51·8 ± 0·6 (2)
	41	47·7 ± 2·4 (3)	22·2 ± 0·6 (3)	33·3 ± 5·2 (3)	45·6 ± 1·6 (2)
	49	42·6 ± 0·2 (2)	18·0 (1)	26·1 ± 9·0 (3)	43·2 ± 1·6 (2)
Coprinus	0	251·3 ± 4·4 (2)	246·7 ± 19·2 (4)	252·8 ± 0 (2)	240·6 ± 18·4 (4)
	3	175·5 ± 22·4 (3)	173·9 ± 50·6 (6)	159·4 ± 0·9 (3)	167·2 ± 50·3 (5)
	6	145·7 ± 5·1 (3)	27·2 ± 4·6 (5)	112·3 ± 2·1 (3)	101·8 ± 49·1 (5)
	9	71·5 ± 8·5 (3)	25·3 ± 11·4 (6)	61·3 ± 4·7 (3)	58·7 ± 29·2 (5)
	12	37·3 ± 5·9 (3)	17·5 ± 2·9 (6)	27·5 ± 3·8 (3)	23·3 ± 8·6 (6)
	15	22·3 ± 5·2 (3)	15·2 ± 2·3 (6)	17·7 ± 2·4 (3)	18·1 ± 4·8 (5)
Volvariella	0	302·6 ± 1·1 (2)	275·1 ± 24·9 (2)	304·2 ± 5·4 (2)	282·0 ± 8·7 (2)
	4	269·6 ± 5·5 (3)	260·0 ± 16·1 (3)	272·2 ± 11·8 (3)	252·8 ± 4·8 (3)
	9	263·2 ± 12·1 (3)	256·8 ± 17·8 (3)	261·2 ± 16·1 (3)	260·5 ± 5·2 (3)
	14	143·1 ± 6·1 (3)	71·7 ± 8·5 (3)	169·3 ± 3·2 (3)	157·2 ± 4·7 (3)
	19	61·9 ± 6·1 (3)	39·4 ± 3·4 (3)	61·3 ± 5·4 (3)	67·9 ± 7·3 (3)
	24	26·8 ± 3·4 (3)	37·8 ± 5·6 (3)	26·7 ± 3·0 (3)	27·0 ± 3·1 (3)

Entries in the table are the means ± S.E. for assays of the number of replicate cultures shown in parentheses. The initial concentration of protein (casein) is shown by the 0 days growth samples which are assays of uninoculated medium. The flasks contained 25 ml of medium supplemented with protein alone, protein + 1% glucose, protein + 30 mM-NH_4Cl, or protein + 0·05% $MgSO_4$.

the addition of glucose caused a small but significant acceleration of the rate of protein removal from the medium; ammonium or sulphate supplementation had no effect (Table 1).

Glucose utilization

In media supplemented with protein, *A. bisporus* cultures completely utilized the supplied glucose by about the 25th day of incubation, and utilization was unaffected by further supplementation with ammonium or sulphate. In the absence of protein only about 50% of the glucose was used (Table 2). In media lacking a nitrogen or sulphur source, 30% of the glucose was removed from the medium by day 25 but was subsequently returned to the medium.

In Treschow medium supplemented with protein, glucose utilization by *C. cinereus* was unaffected by addition of ammonium or sulphate, being completely utilized by day 12 (Table 2). This was a rather greater rate of utilization than was observed in the absence of protein, i.e. in glucose–ammonium–sulphate medium, when only about 80% of the glucose was utilized by day 12. In the absence of a sulphur source, 50% of the glucose was utilized in the first three days, again implying effective sulphur-scavenging during this time. Some glucose utilization was also observed in media deficient in nitrogen. Some strains of *C. cinereus* are known to respond to nitrogen starvation by derepressing enzymes involved in ammonia scavenging (Stewart & Moore, 1974; Moore, 1981).

In *V. volvacea* cultures grown in media supplemented with protein, 40% of the supplied glucose was utilized in the first 9 d, with complete utilization by day 14, in the presence of additional ammonium; without additional ammonium supplementation glucose was not completely utilized until day 19 (Table 2). In the absence of protein (= glucose–ammonium–sulphate medium) 40% of the glucose was utilized in the first 14 d with no subsequent removal from the medium. In sulphur-deficient media the glucose concentration decreased considerably, revealing an ability to scavenge and re-utilize sulphur from the inoculum. In media devoid of a nitrogen source, very little glucose was removed from the medium.

Concentration of ammonia in the medium

Changes in the concentration of ammonia in the medium of cultures during incubation are summarized in Table 3. For all three organisms it can be generalized that in all media supplemented with

Table 2. *Amount of residual substrate glucose (mg per flask) left in the medium during incubation of cultures of* Agaricus, Coprinus *and* Volvariella

Organism	Days growth	Supplements to the medium: Glucose + ammonium + sulphate	Protein + glucose	Protein + glucose + ammonium	Protein + glucose + sulphate	Protein + glucose + ammonium + sulphate	Glucose + ammonium (S-deficient)
Agaricus	0	259·1 ± 5·7 (2)	223·4 ± 8·8 (2)	224·2 ± 1·2 (2)	202·9 ± 15·2 (2)	218·5 ± 2·8 (2)	259·1 ± 3·3 (2)
	9	232·7 ± 9·8 (3)	150·5 ± 18·4 (3)	159·5 ± 12·0 (2)	187·0 ± 14·1 (2)	177·5 ± 9·3 (2)	210·4 ± 14·9 (3)
	17	166·1 ± 10·6 (3)	89·8 ± 0·4 (2)	113·9 ± 7·7 (3)	89·9 ± 20·4 (3)	105·3 ± 8·5 (3)	191·8 ± 32·4 (3)
	25	122·6 ± 12·2 (3)	1·4 ± 1·6 (3)	12·9 ± 6·8 (2)	30·6 ± 35·0 (3)	43·1 ± 20·1 (3)	174·7 ± 9·5 (3)
	33	124·1 ± 15·4 (3)	0	0	0	0	212·5 ± 6·4 (3)
	41	114·7 ± 4·3 (3)	0	0	0	0	225·9 ± 25·4 (3)
	49	118·0 ± 4·2 (3)	0	0	0	0	239·8 ± 1·4 (3)
Coprinus	0	254·3 ± 34·2 (4)	245·8 ± 16·5 (4)	236·7 ± 19·2 (4)	245·8 ± 25·0 (4)	252·9 ± 15·2 (2)	253·1 ± 35·9 (4)
	3	171·1 ± 29·6 (6)	176·6 ± 27·3 (6)	176·1 ± 11·9 (3)	183·0 ± 5·9 (6)	142·8 ± 37·0 (3)	134·6 ± 48·8 (6)
	6	114·6 ± 30·4 (6)	96·7 ± 30·2 (5)	77·1 ± 36·4 (5)	128·2 ± 10·7 (6)	109·8 ± 14·1 (3)	122·2 ± 31·9 (6)
	9	not done	28·2 ± 30·3 (6)	33·9 ± 19·7 (4)	52·0 ± 28·9 (5)	42·4 ± 6·7 (3)	113·9 ± 29·6 (6)
	12	49·1 ± 4·3 (4)	0·3 ± 0·3 (6)	0·3 ± 0·5 (3)	0·8 ± 0·7 (6)	0·4 ± 0·5 (3)	136·3 ± 50·1 (5)
	15	118·4 ± 63·6 (4)	0	0	0	0	149·4 ± 51·8 (3)
Volvariella	0	264·1 ± 2·0 (2)	235·9 ± 4·9 (2)	267·7 ± 4·4 (2)	252·1 ± 5·8 (2)	256·0 ± 9·2 (2)	265·9 ± 9·0 (2)
	4	260·0 ± 12·0 (3)	195·5 ± 5·3 (3)	215·3 ± 2·8 (3)	167·9 ± 19·9 (3)	207·3 ± 10·6 (3)	228·7 ± 9·6 (2)
	9	183·8 ± 21·1 (3)	140·6 ± 4·1 (3)	170·3 ± 10·2 (3)	134·8 ± 5·4 (3)	152·7 ± 2·9 (3)	220·4 ± 12·4 (2)
	14	89·3 ± 3·0 (3)	13·1 ± 1·4 (3)	36·9 ± 5·2 (2)	23·7 ± 7·9 (3)	48·5 ± 13·8 (3)	92·7 ± 2·4 (2)
	19	88·8 ± 11·7 (3)	4·9 ± 2·6 (3)	3·0 ± 1·0 (3)	1·6 ± 0·4 (3)	2·2 ± 0·4 (3)	95·8 ± 6·6 (2)
	24	77·9 ± 6·8 (3)	2·0 ± 0·2 (3)	2·6 ± 0·4 (3)	2·8 ± 0·5 (3)	3·7 ± 0·5 (3)	53·0 ± 3·7 (2)

Entries in the table are the means ± S.E. for assays of the number of replicate cultures shown in parentheses. The initial concentration of glucose is shown by the 0 days growth samples which are assays of uninoculated medium. The flasks contained 25 ml of medium supplemented with 1% glucose + 30 mM-NH_4Cl + 0·05% $MgSO_4$, protein (1% casein) + 1% glucose, protein + glucose + 30 mM-NH_4Cl, protein + glucose + 0·5% $MgSO_4$, protein + glucose + $MgSO_4$ + NH_4Cl, or a protein-free medium containing glucose and ammonium but deficient in sulphur.

protein (whether or not initially supplemented with 30 mM-NH_4Cl), ammonia levels in the medium increased during incubation of the culture. Only when ammonium was the sole source of nitrogen did the ammonium content of the medium decline during incubation.

pH of medium during incubation

Although differences were observed in the scales of the changes and, as a consequence of the differing growth rates, in the rates with which the changes occurred, generally for all three organisms the medium pH became more acid during growth on media containing glucose as a carbon source (whether or not protein was also present) and became more alkaline in media with protein as sole carbon source.

DISCUSSION

In media supplemented with both protein and glucose as carbon sources, the two nutrient sources were utilized simultaneously. Similar observations have been made with *A. bisporus* grown on bacteria and either glucose or cellulose as carbon sources (D. A. Wood & T. R. Fermor, unpubl.), and with white rot fungi, such as *Sporotrichum pulverulentum* Novobranova (Ander & Eriksson, 1977) and *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. (Hiroi & Eriksson, 1976) grown on lignin and a polysaccharide. This mechanism appears to differ from that in other micro-organisms, especially the bacteria where macromolecules are believed to be degraded only on exhaustion of the easily metabolizable carbon sources (Engelking & Seidler, 1974; Wouters & Bieysman, 1977). Such a contrast presumably reflects different ecophysiological strategies such that the slower rate of multiplication of these basidiomycetes is coupled with much more efficient resource utilization.

Medium pH decreased in the early stages of growth of the organisms on media supplemented with glucose. This decrease in pH may well have been caused by mechanisms related to glucose utilization, such as proton pumping linked to sugar uptake or excretion of metabolic by-products. Subsequent alkalinization in media containing supplied protein paralleled increases in ammonia concentrations in the medium; excretion of ammonium may also be an expression of proton pumping. Similar responses in medium pH have been observed with other organisms grown in the presence of glucose (Kimura & Tsuchiya, 1982). In the absence of protein the pH decreased rapidly in

Table 3. *Concentration of ammonia (μmole per flask) in the media of cultures of* Agaricus, Coprinus *and* Volvariella

Organism	Days growth	Supplements to the medium: Protein	Protein + glucose	Protein + ammonium	Protein + glucose + ammonium	Glucose + ammonium + sulphate
Agaricus	0	15·0±0 (2)	19·0±2·8 (2)	856·5±7·8 (2)	826·5±20·5 (2)	815·0±0 (2)
	9	72·3±3·5 (3)	12·3±1·5 (3)	981·7±27·1 (3)	861·5±26·2 (2)	926·7±42·0 (3)
	17	243·0±27·8 (3)	95·5±16·3 (2)	1165·0±17·0 (2)	814·7±39·0 (3)	675·0±16·1 (3)
	25	286·0±1·4 (2)	277·7±31·0 (3)	1380·7±15·2 (3)	968·5±6·4 (2)	684·7±42·0 (3)
	33	399·0±11·5 (3)	707·7±76·7 (3)	1381·3±88·0 (3)	1279·0±32·7 (3)	661·3±22·8 (3)
	41	533·0±8·5 (3)	882·7±18·7 (3)	1388·3±36·1 (3)	1424·0±62·2 (2)	639·7±32·0 (3)
	49	560·5±16·3 (2)	1029 (1)	1471·7±42·9 (3)	1522·0±36·1 (3)	717·7±15·9 (3)
Coprinus	0	31·7±18·1 (6)	7·3±3·3 (4)	773·2±64·3 (6)	627·2±37·6 (6)	560·0±80·3 (4)
	3	34·0±4·5 (8)	4·3±2·9 (6)	685·9±101·6 (8)	784·5±130·3 (6)	561·3±133·2 (6)
	6	178·8±28·3 (8)	154·2±144·3 (5)	841·4±71·6 (8)	483·5±130·3 (8)	351·5±60·8 (6)
	9	343·7±81·8 (7)	454·5±61·2 (6)	866·5±184·9 (8)	534·4±291·8 (7)	392·7±38·3 (3)
	12	568·6±60·0 (8)	820·8±39·2 (6)	1121·4±199·2 (8)	485·7±94·2 (6)	301·3±100·4 (4)
	15	782·0±99·7 (8)	957·0±121·1 (6)	1177·4±196·5 (8)	1122·6±286·3 (7)	423·5±46·9 (4)
Volvariella	0	13·8±0·5 (4)	11·8±3·2 (4)	1087·5±35·7 (4)	1080·8±22·1 (4)	1077·5±5·4 (2)
	4	75·5±3·4 (6)	62·2±31·0 (6)	1040·8±16·5 (6)	1125·5±244·3 (6)	1016·2±30·7 (3)
	9	107·2±7·3 (6)	4·0±1·7 (6)	1109·2±21·0 (6)	977·5±95·9 (6)	899·9±90·7 (3)
	14	358·3±23·5 (6)	71·3±37·6 (6)	1228·0±75·3 (6)	809·2±64·2 (6)	789·4±8·9 (3)
	19	1042·7±211·4 (6)	346·0±88·2 (6)	1859·7±93·7 (6)	1006·5±279·5 (6)	750·4±45·0 (3)
	24	1474·7±147·7 (6)	983·8±63·8 (6)	2004·7±185·6 (6)	1395·0±202·3 (6)	720·7±17·0 (3)

Entries in the table are the means ± S.E. for assays of the number of replicate cultures shown in parentheses. The initial concentration of ammonia is shown by the 0 days growth samples which are assays of uninoculated medium. The flasks contained 25 ml of medium supplemented with protein alone, protein + 1% glucose, protein + 30 mM-NH_4Cl, protein + 1% glucose + 30 mM-NH_4Cl, or a protein-free medium containing 1% glucose + 30 mM-NH_4Cl + 0·05% $MgSO_4$.

the early stages of growth and subsequently remained acidic. Glucose utilization was incomplete in these cultures so the very acidic pH values attained probably inhibited further growth of the organisms.

In the presence of both carbon sources utilization of protein and glucose was more rapid than when either was present as sole source of carbon. The increased rate and efficiency of utilization of both carbon sources obviously contributed to the 3–4-fold increase in biomass of mycelia grown in media supplemented with protein and glucose, than with one carbon source.

The organisms appeared to utilize the protein as effectively as glucose when used as sole source of carbon. Protein 'utilization' was only measured here in terms of loss of TCA-precipitable material from the medium; we have no direct measure of assimilation of protein degradation products by the mycelia. However, the excretion of large quantities of ammonia into the medium by all three species demonstrates that considerable proportions of the supplied protein were assimilated. Growth and ammonium utilization from protein-free medium, i.e. glucose + ammonium + sulphate, gives a measure of the N-requirement of these species, and at about 0·05 mg N/mg D.W. for both *Agaricus* and *Coprinus* and 0·08 mg N/mg D.W. in *Volvariella* this is consonant with known values for elemental composition of fungi. Yet on medium containing protein as sole supplement *Agaricus* produced about 40 mg D.W. of mycelium and 160 mg of supplied protein was 'utilized'. Making the usual presumption that protein contains 16% by weight of nitrogen, this amount of protein is equivalent to some 26 mg of nitrogen; far more than required by the biomass of mycelium formed. Significantly, these same cultures excreted almost 8 mg (540 μmol) of ammonia-N into the medium. The corresponding conversion values for *Coprinus* and *Volvariella* were respectively (in round figures) 37 mg protein-N used/11 mg ammonia-N excreted and 43 mg protein-N used/20 mg ammonia-N excreted. Thus in these cultures one-third to one-half of the supplied protein must have been assimilated and metabolized to ammonia during its use as a carbon source. The excretion route is presumably via the urea cycle, which has been shown to operate in both *Coprinus* (Al-Gharawi & Moore, 1977) and *Agaricus* (Levenberg, 1962).

Excretion of large quantities of ammonia by *Agaricus* was unexpected since high concentrations of gaseous ammonia have been reported to inhibit growth of this organism (O'Donoghue, 1965). Inspection of Table 3 shows that these conversions were little influenced by further supplementation of the medium either with glucose or, more significantly, with ammonium. Thus neither of these primary metabolites had any catabolic repressive effect on protein utilization.

All three species tested are thus able to utilize protein as a source of C, N and S and are effectively free of catabolite repression of this capability. It is evident in Table 3, though, that *Volvariella* excreted less ammonia into the medium when this contained protein + glucose than when protein was present as sole carbon source. As there was no corresponding reduction in removal of TCA-precipitable protein from such cultures (Table 1) this may indicate that in this species glucose partially represses the uptake or utilization of amino acids or peptides.

H. M. K. acknowledges receipt of a SERC-CASE Studentship during the course of this work.

REFERENCES

AL-GHARAWI, A. & MOORE, D. (1977). Factors affecting the amount and the activity of the glutamate dehydrogenases of *Coprinus cinereus*. *Biochimica et Biophysica Acta* **496**, 95–102.

ANDER, P. & ERIKSSON, K.-E. (1977). Selective degradation of wood components by white rot fungi. *Physiologia Plantarum* **41**, 239–248.

EDDY, B. P. & JACOBS, L. (1976). Mushroom compost as a source of food for *Agaricus bisporus*. *Mushroom Journal* **38**, 56–67.

ENGELKING, H. M. & SEIDLER, R. J. (1974). The involvement of extracellular enzymes in the metabolism of *Bdellovibrio*. *Archives of Microbiology* **95**, 293–304.

FERMOR, T. R. & WOOD, D. A. (1981). Degradation of bacteria by *Agaricus bisporus* and other fungi. *Journal of General Microbiology* **126**, 377–387.

GERRITS, J. P. G. (1969). Organic compost constituents and water utilised by the cultivated mushroom during spawn run and cropping. *Mushroom Science* **7**, 111–126.

HIROI, T. & ERIKSSON, K.-E. (1976). Influence of cellulose on degradation of lignins by the white rot fungus *Pleurotus ostreatus*. *Svensk pappers-tidning* **79**, 157–161.

KIMURA, T. & TSUCHIYA, K. (1982). Characteristics of protease production by *Cephalosporium* species. *Applied and Environmental Microbiology* **43**, 654–658.

LEVENBERG, B. (1962). Role of L-glutamine as donor of carbamyl nitrogen for the enzymatic synthesis of citrulline in *Agaricus bisporus*. *Journal of Biological Chemistry* **237**, 2590–2598.

MOORE, D. (1981). Evidence that the NADP-linked glutamate dehydrogenase of *Coprinus cinereus* is regulated by acetyl-CoA and ammonium levels. *Biochimica et Biophysica Acta* **661**, 247–254.

O'DONOGHUE, D. C. (1965). Relationship between some compost factors and their effects on the yield of *Agaricus*. *Mushroom Science* **9**, 245–254.

QUIMIO, T. H. (1981). Nutritional studies on *Volvariella volvacea*. *Mushroom Newsletter for the Tropics* **2**, 9–13.

SPARLING, G. P., FERMOR, T. R. & WOOD, D. A. (1982). Measurement of the microbial biomass in composted wheat straw, and the possible contribution of the biomass to the nutrition of *Agaricus bisporus*. *Soil Biology and Biochemistry* **14**, 609–611.

STANEK, M. (1972). Microorganisms inhabiting mushroom compost during fermentation. *Mushroom Science* **8**, 797–811.

STEWART, G. R. & MOORE, D. (1974). The activities of glutamate dehydrogenase during mycelial growth and sporophore development in *Coprinus lagopus* (*sensu* Lewis). *Journal of General Microbiology* **83**, 73–81.

TRESCHOW, C. (1944). Nutrition of the cultivated mushroom. *Dansk Botanisk Arkiv* **11**, 1–180.

WOOD, D. A. & FERMOR, T. R. (1985). Nutrition of *Agaricus bisporus*. In *The Biology and Technology of the Cultivated Mushroom* (ed. P. B. Flegg, D. M. Spencer & D. A. Wood), pp. 43–61. Chichester: Wiley-Interscience.

WOOD, D. A. & GOODENOUGH, P. W. (1977). Fruiting of *Agaricus bisporus*. Changes in extracellular enzyme activities during growth and fruiting. *Archives of Microbiology* **114**, 161–165.

WOUTERS, J. T. M. & BIEYSMAN, P. J. (1977). Production of some exocellular enzymes by *Bacillus licheniformis* 749/C in chemostat cultures. *FEMS Microbiology Letters* **1**, 109–112.

(*Received for publication 8 November 1985*)